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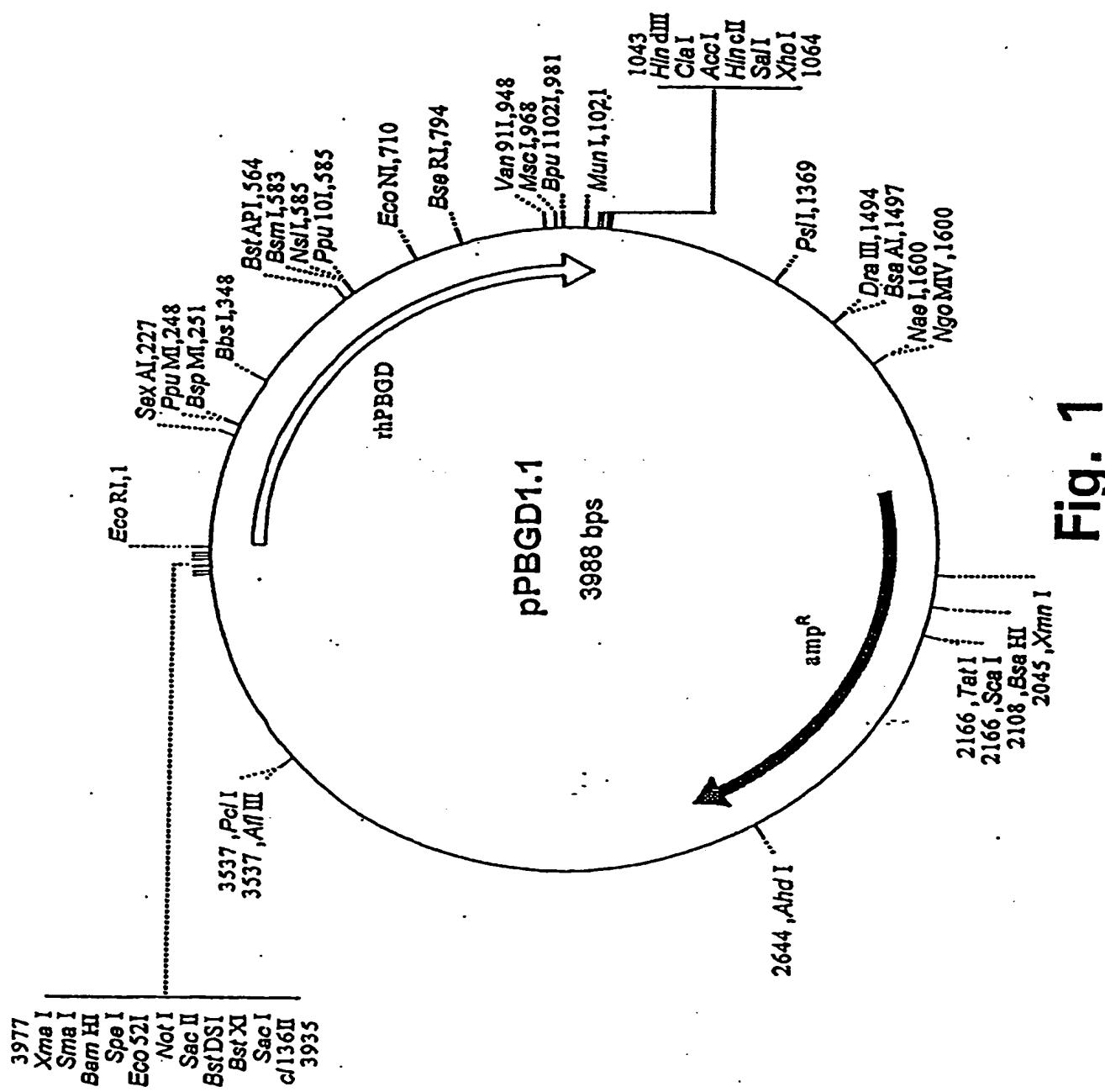
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1/49



2/49

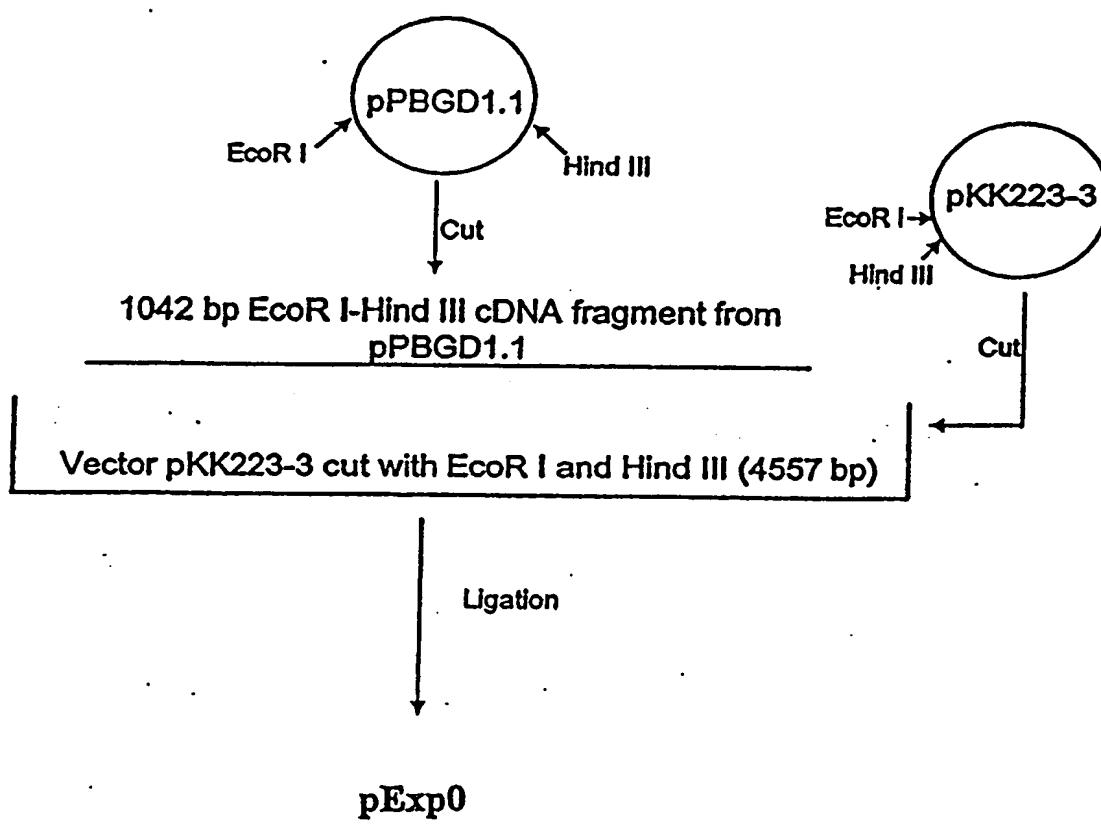


Fig. 2

3/49

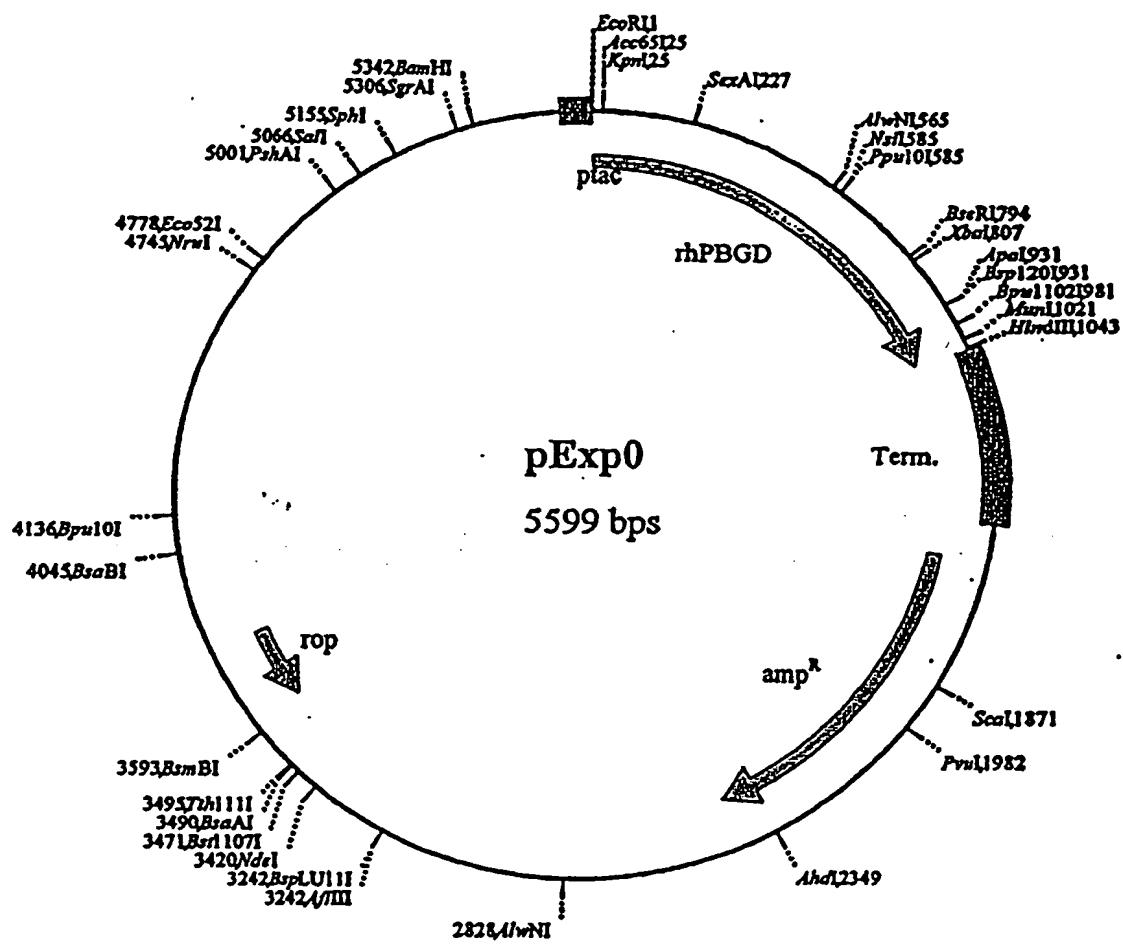


Fig. 3

4/49

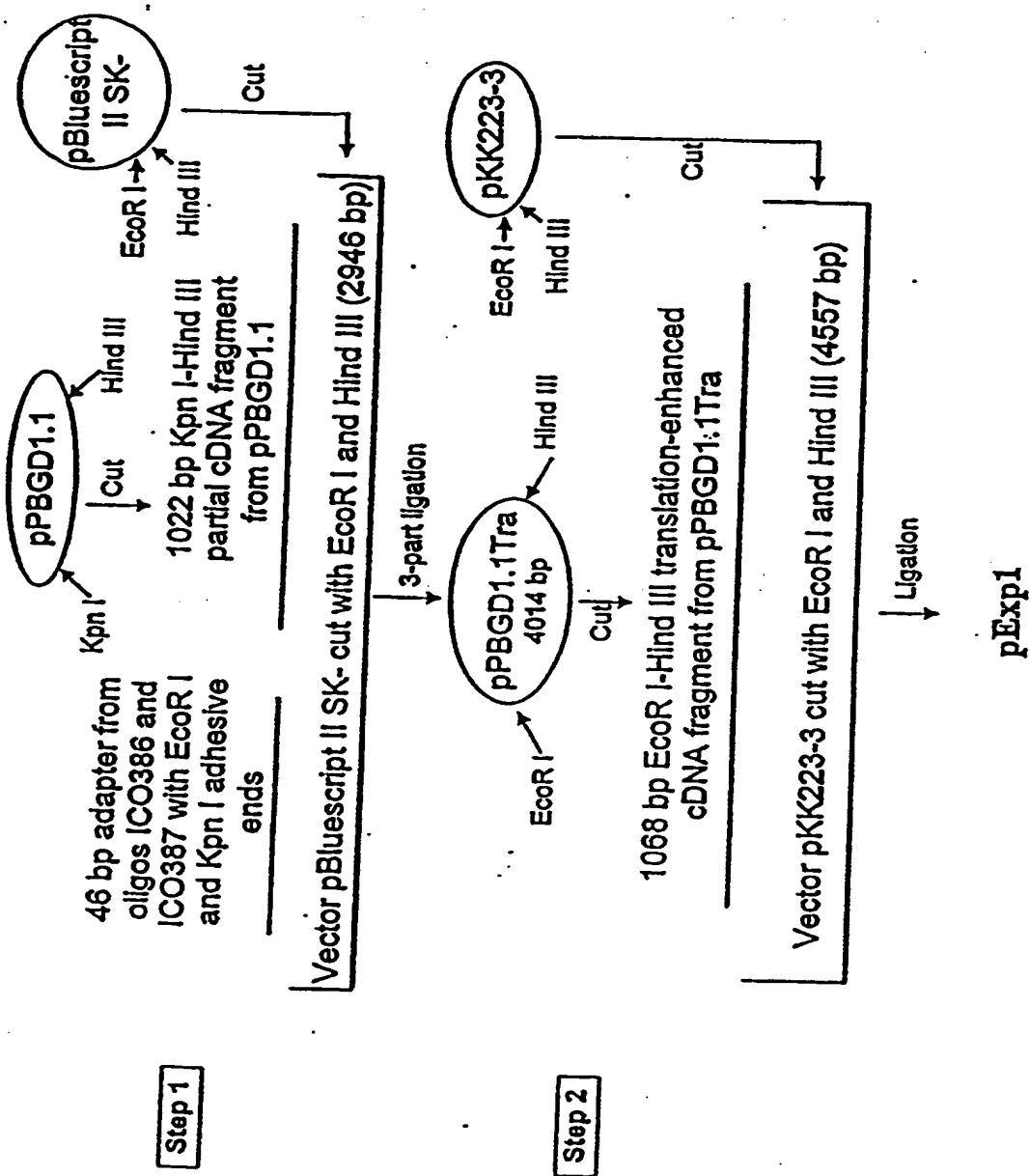


Fig. 4

5/49

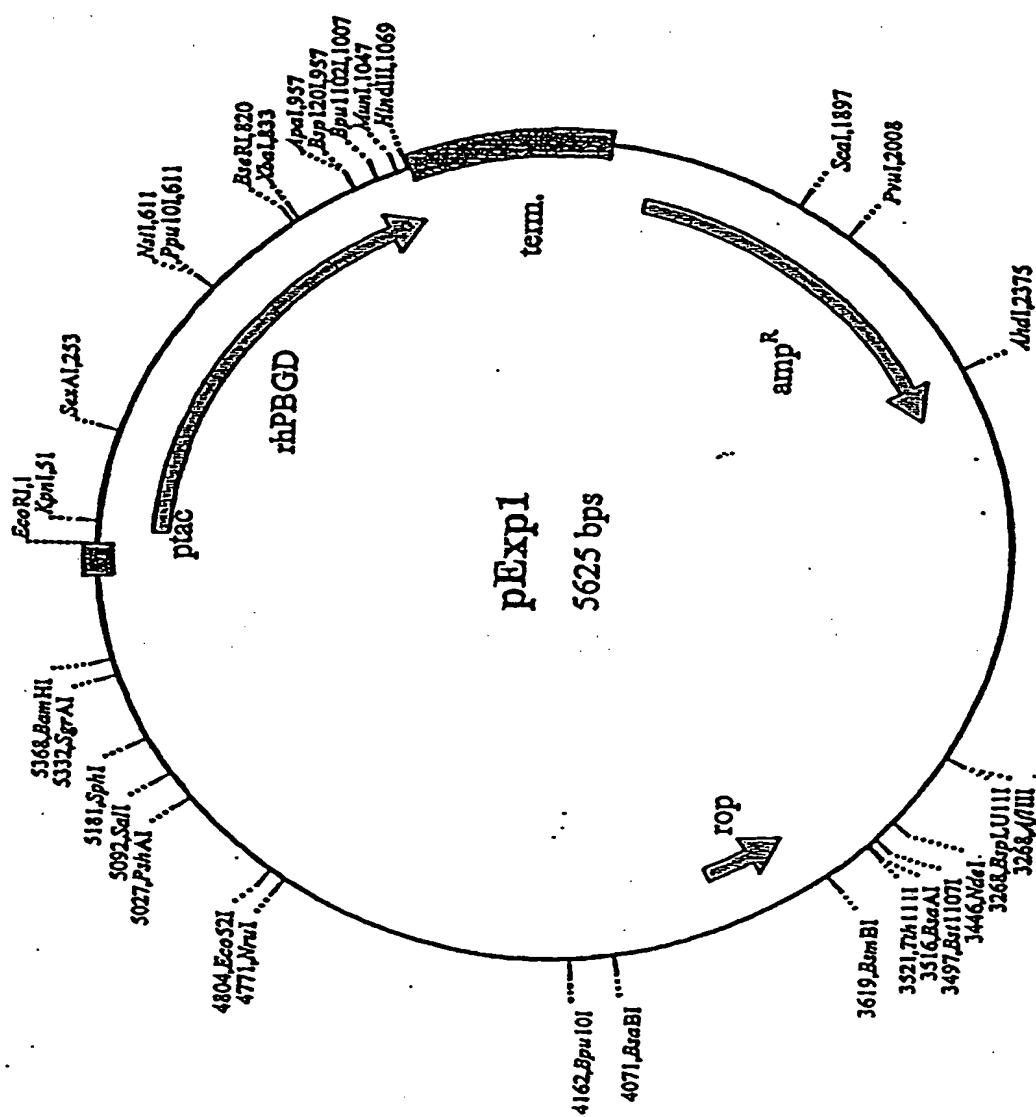


Fig. 5

6/49

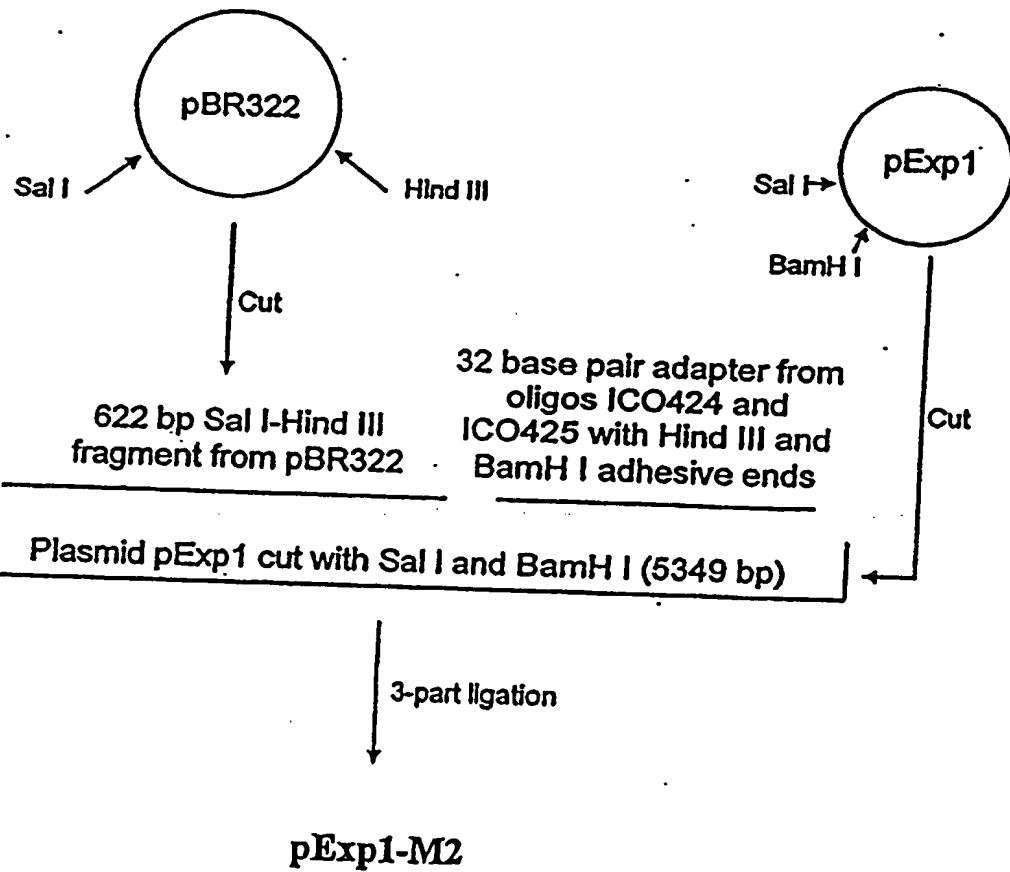


Fig. 6

7/49

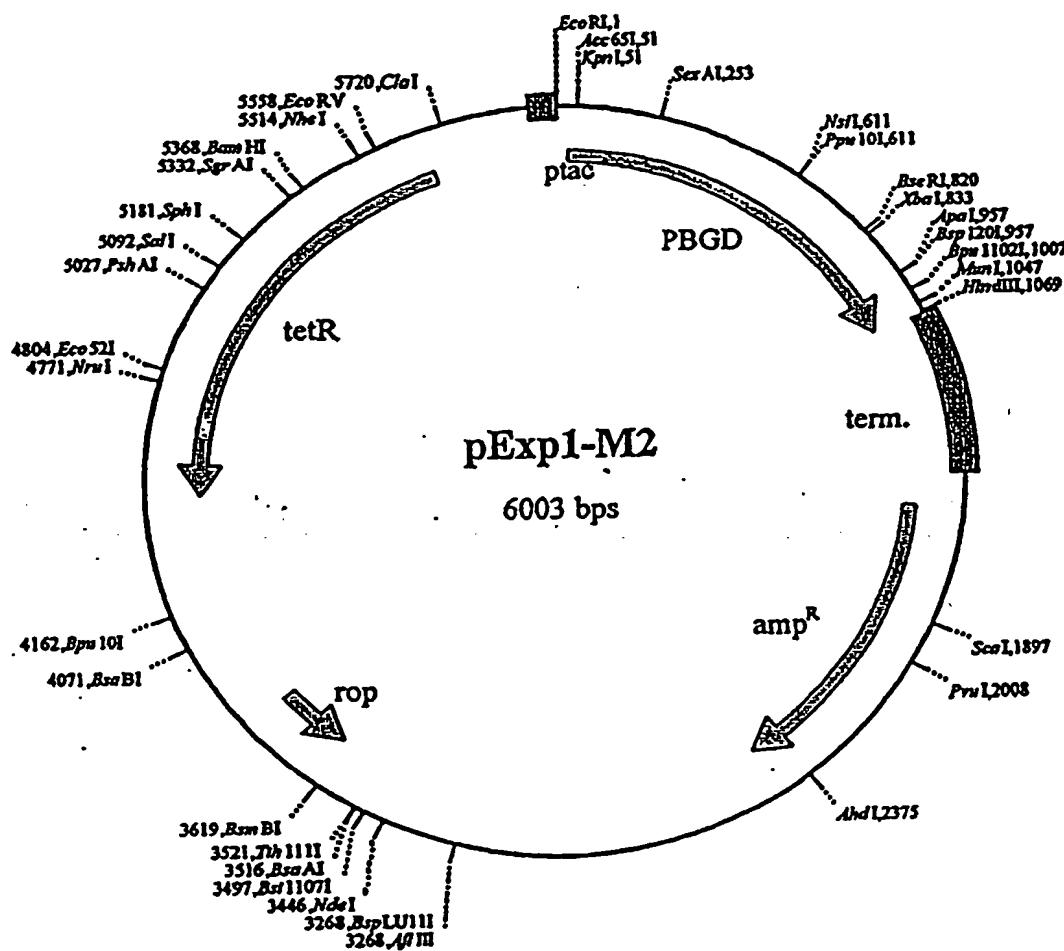
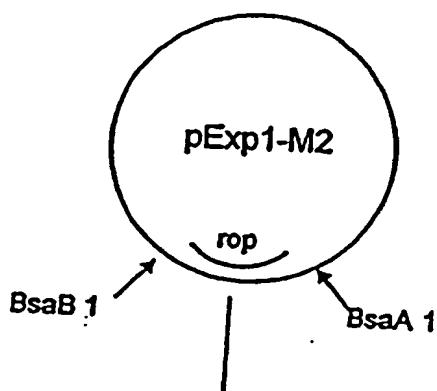


Fig. 7

8/49



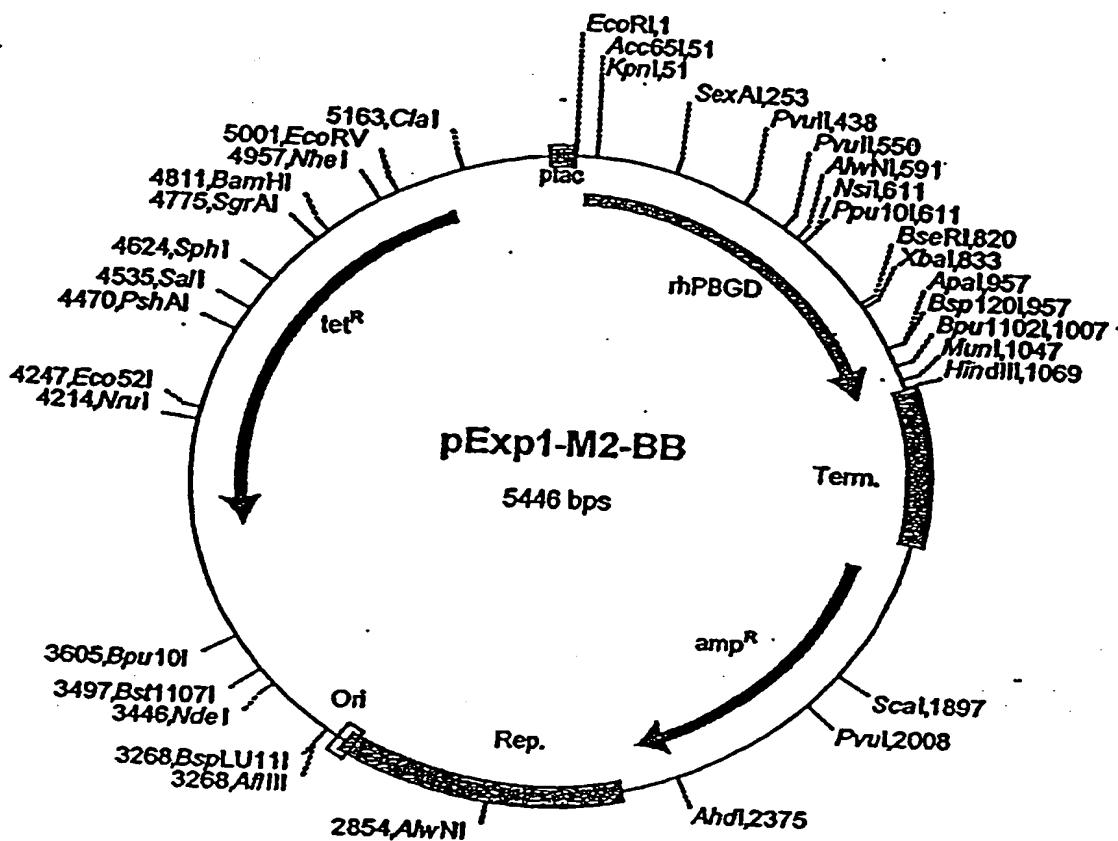
Plasmid pExp1-M2 cut with BsaA I and BsaB I (5446 bp)

Blunt-end ligation

pExp1-M2-BB

Fig. 8

9/49

**PLASMID FEATURES:**

Start	End	Name
33	1067	rhPBGD (open reading frame)
1593	2453	amp ^R (open reading frame)
5105	3915	tet ^R (open reading frame)
5376	1	ptac (promoter)
1075	1501	Term. (terminator region)
3214	2519	Rep. (replication region)
3214		Ori (origin of replication)

Fig. 9

10/49

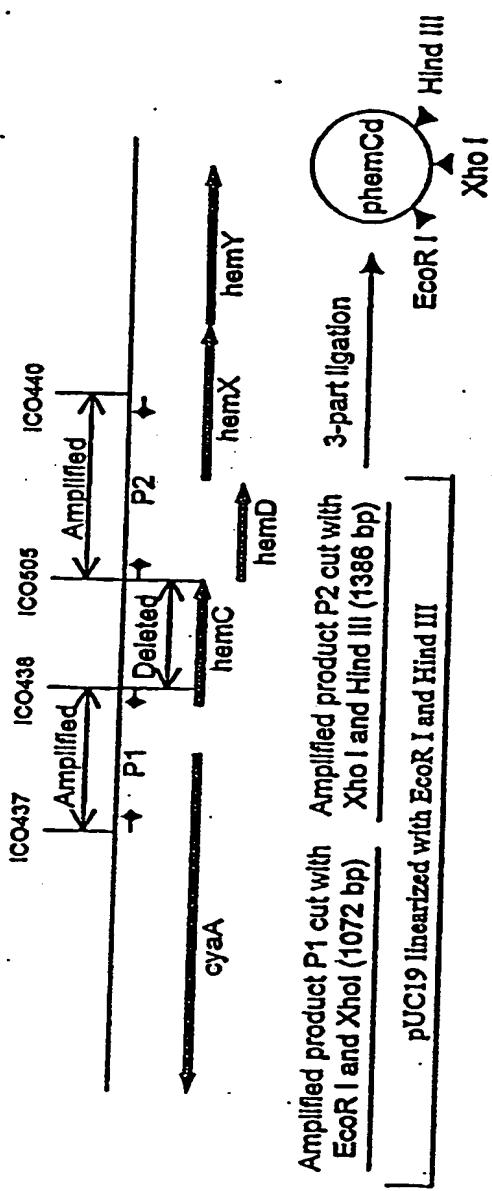


Fig. 10

11/49

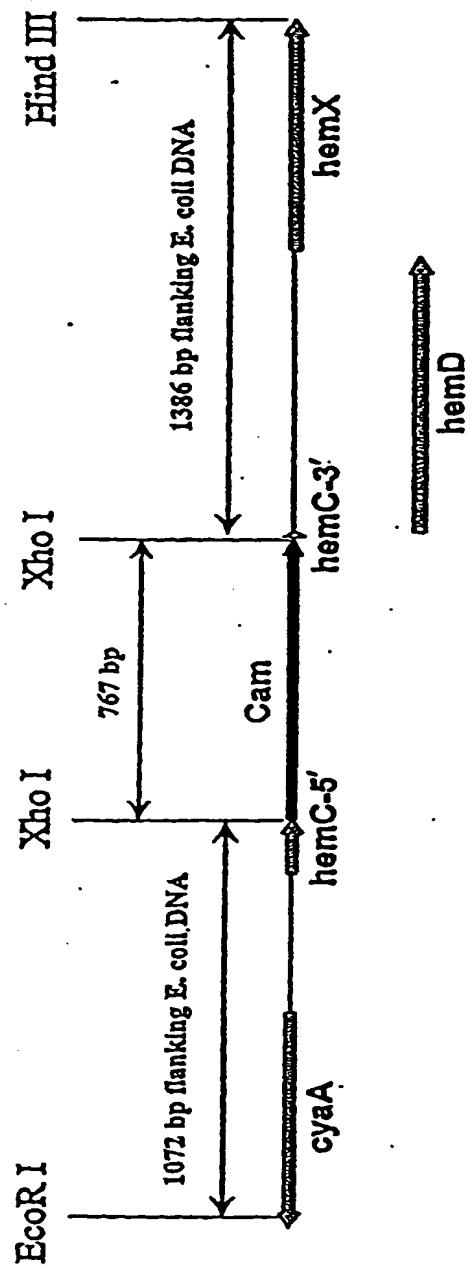


Fig. 11

12/49

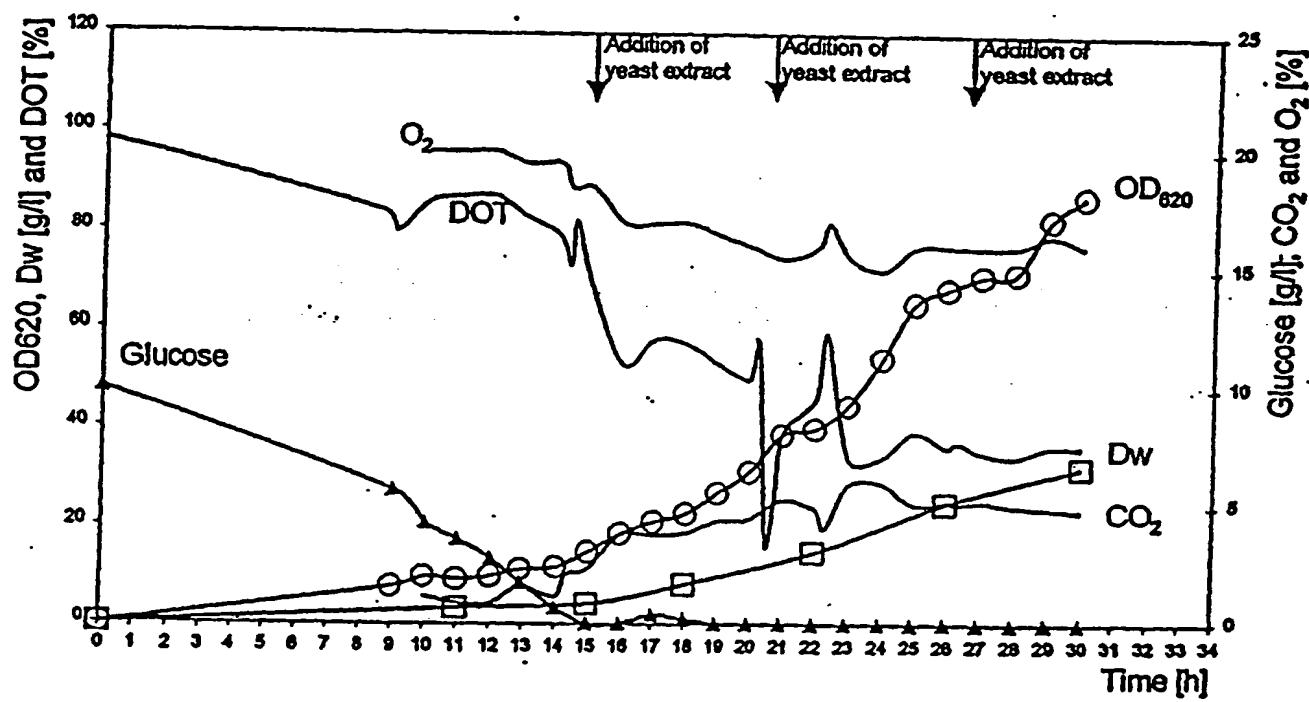


Fig. 12

13/49

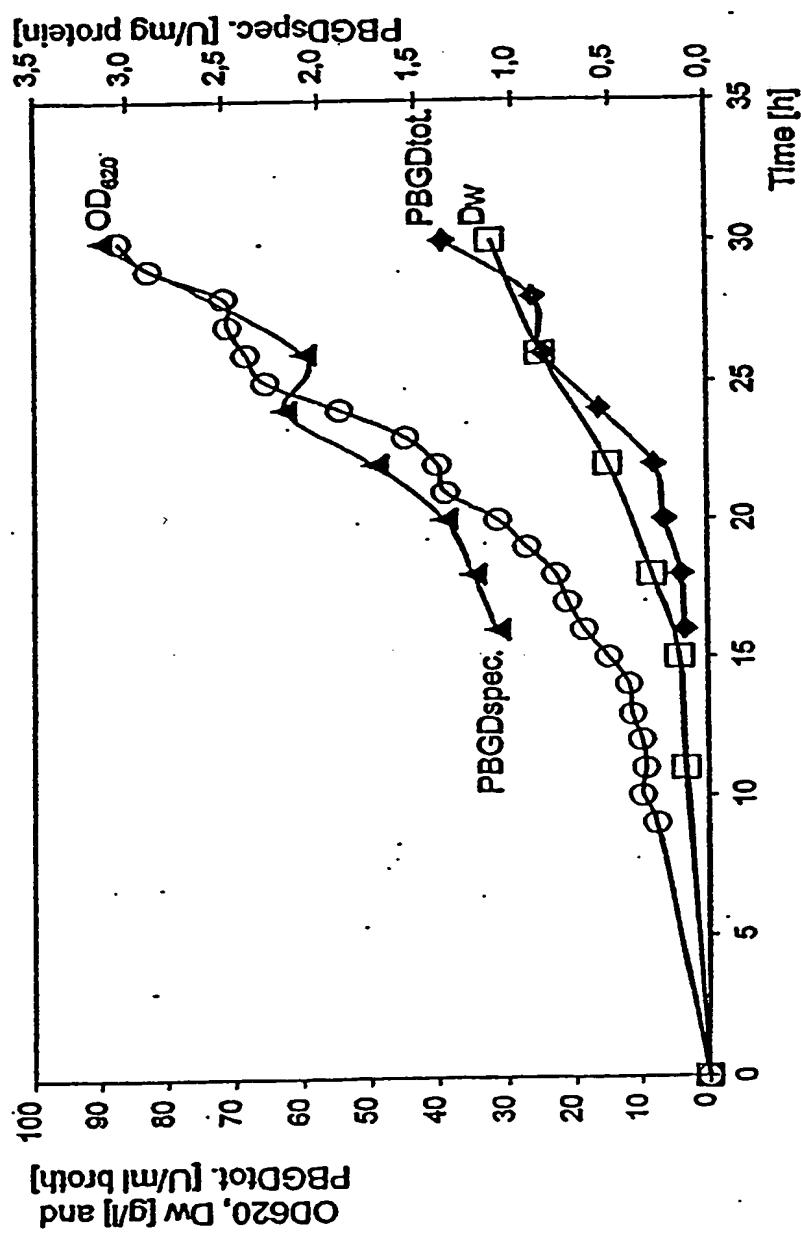
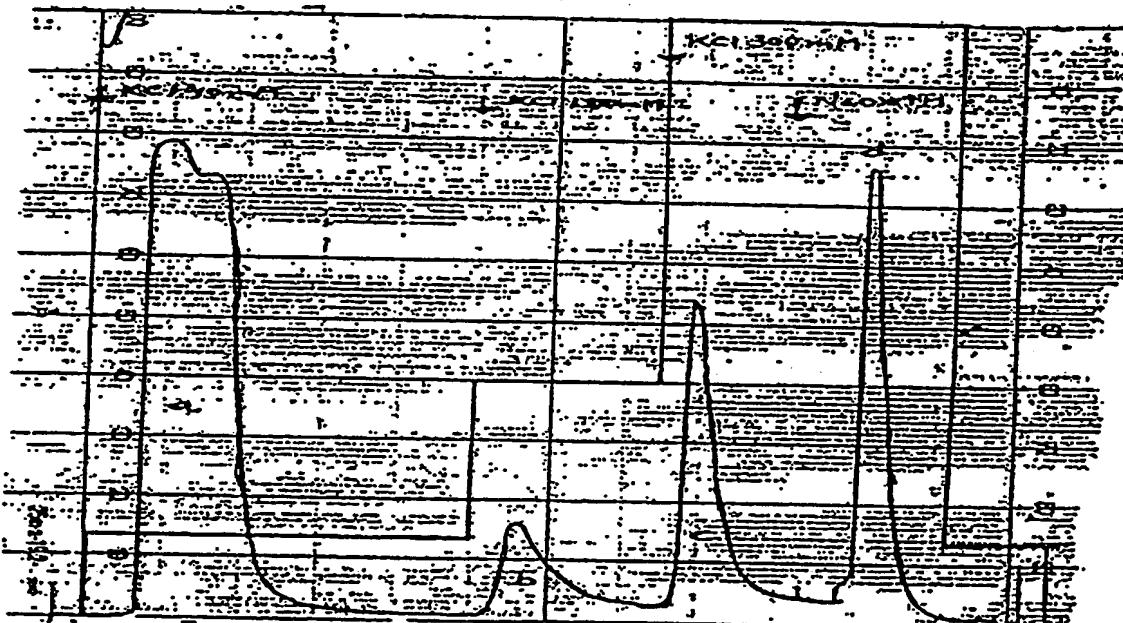


Fig. 13

Fig. 13

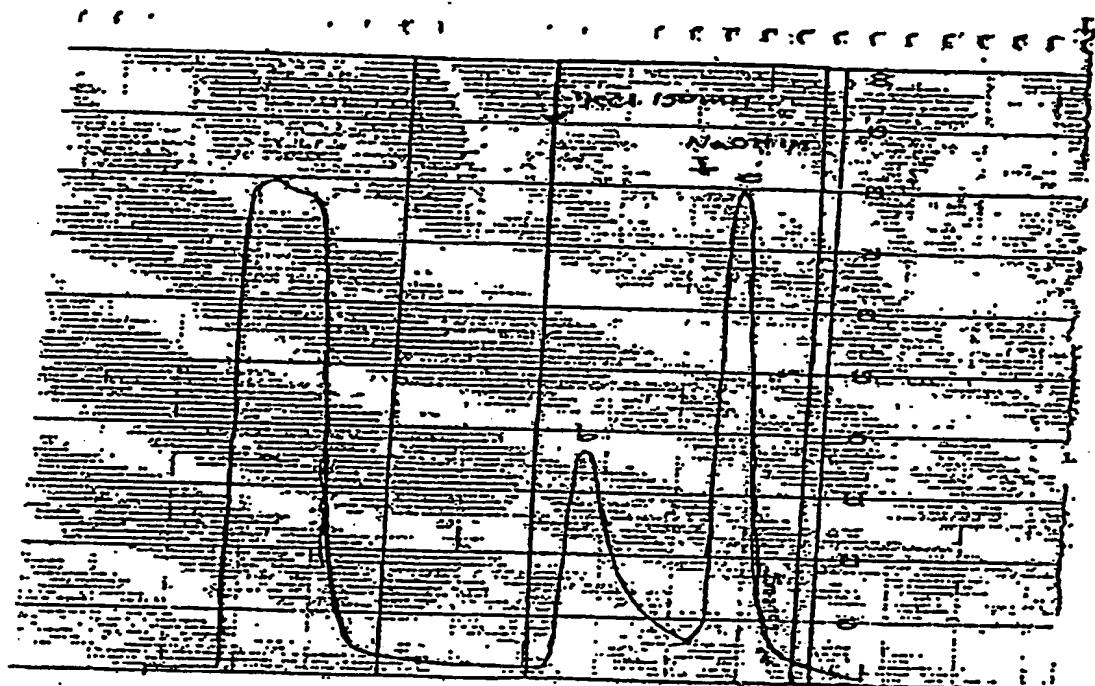
14/49



Chromatography on DEAE-Sepharose FF (DEAE1). Peak a flow through the gel 40 mM KCl, Peak b 120 mM KCl Peak c 300 mM KCl Peak d NaOH 1 M.

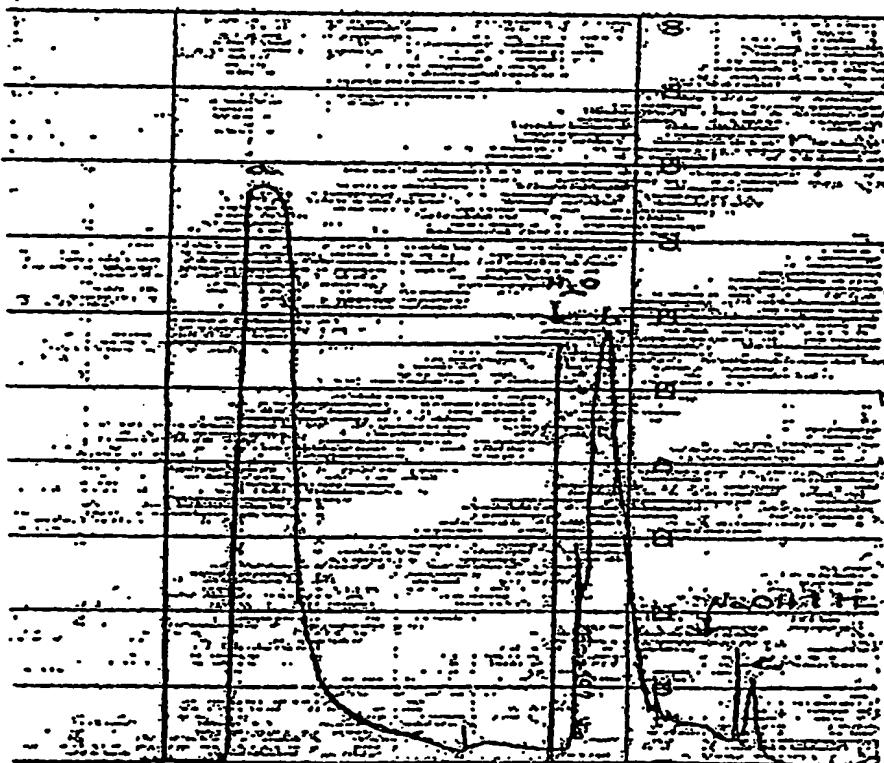
Fig. 14

15/49



Chromatography on DEAE-Sepharose FF (DEAE2). Peak a flow through the gel 40 mM KCl, Peak b 150 mM KCl Peak c NaOH 1 M.

Fig. 15

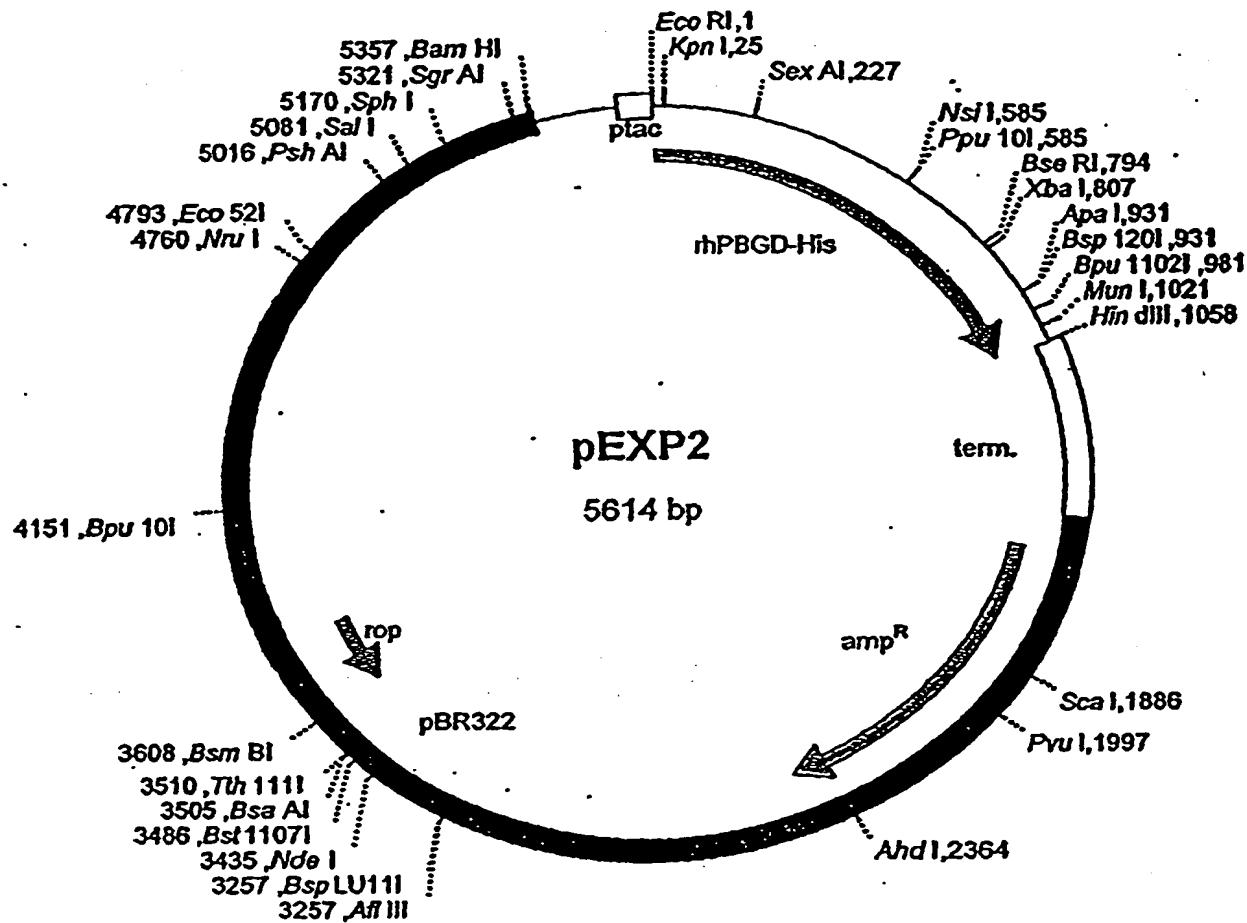
16/49

Chromatography on Butyl-Sepharose 4 FF. Peak a flow through the gel, Peak b water Peak c NaOH 1 M.

Fig. 16

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17/49

**PLASMID FEATURES:**

Start	End	Name
7	1056	rhPBGD-His
1064	1490	terminator region
1582	2442	amp ^R
3820	3629	rop
5362	1490	pBR322
5544	1	ptac (promoter)

Fig. 17

18/49

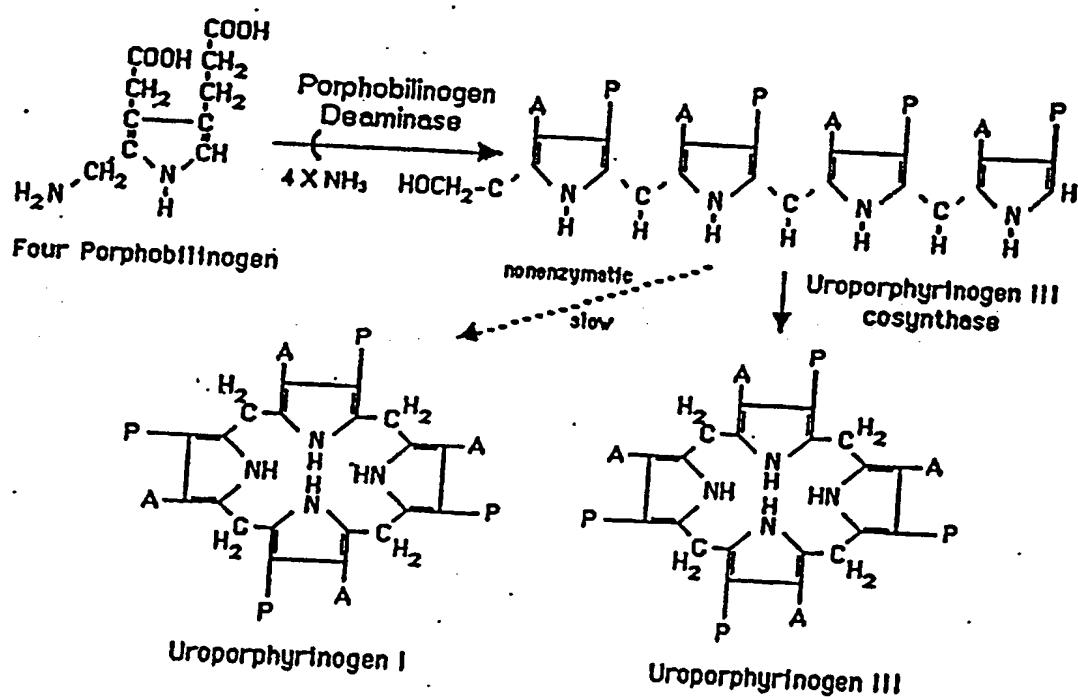


Fig. 18

19/49

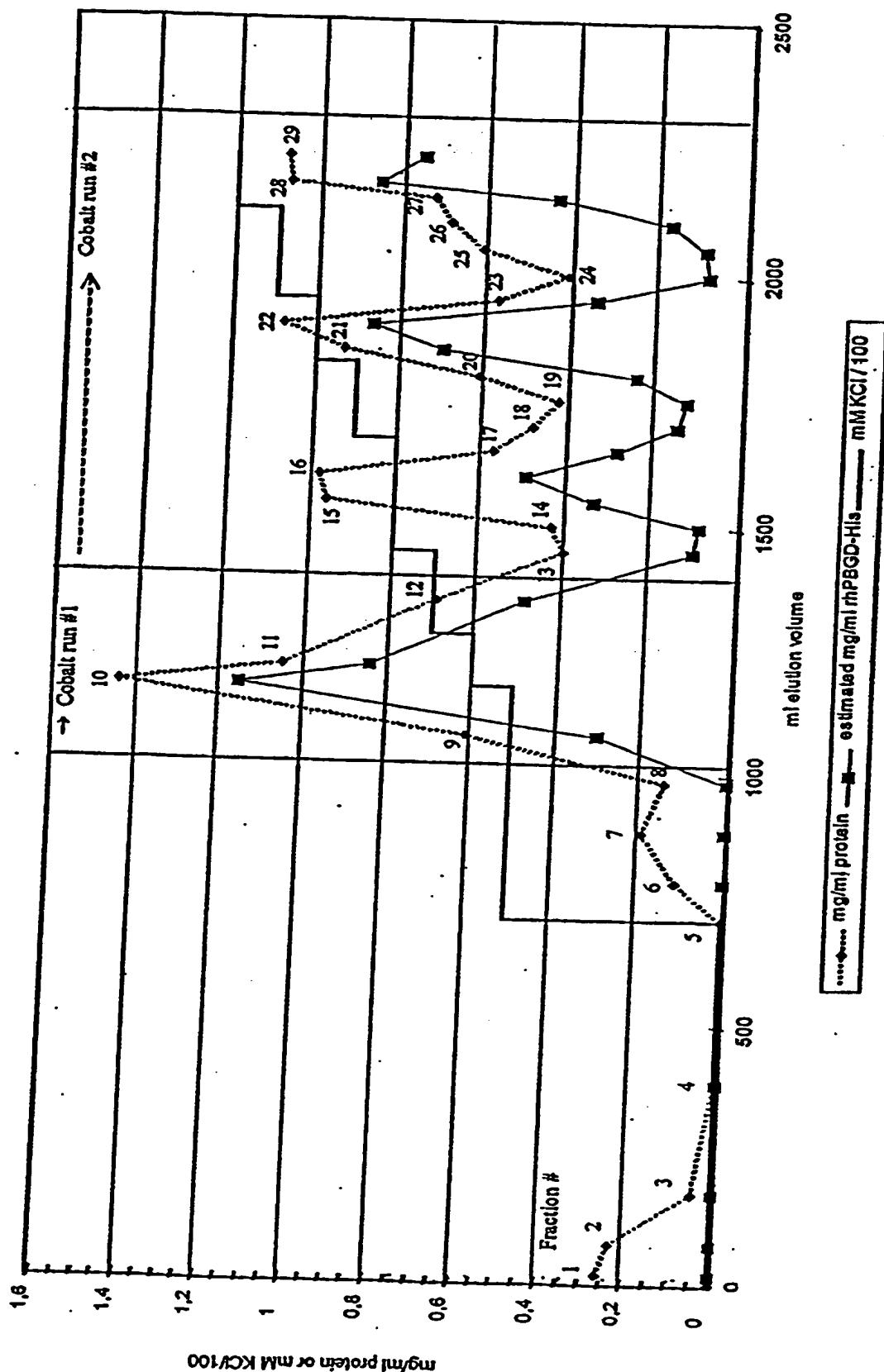
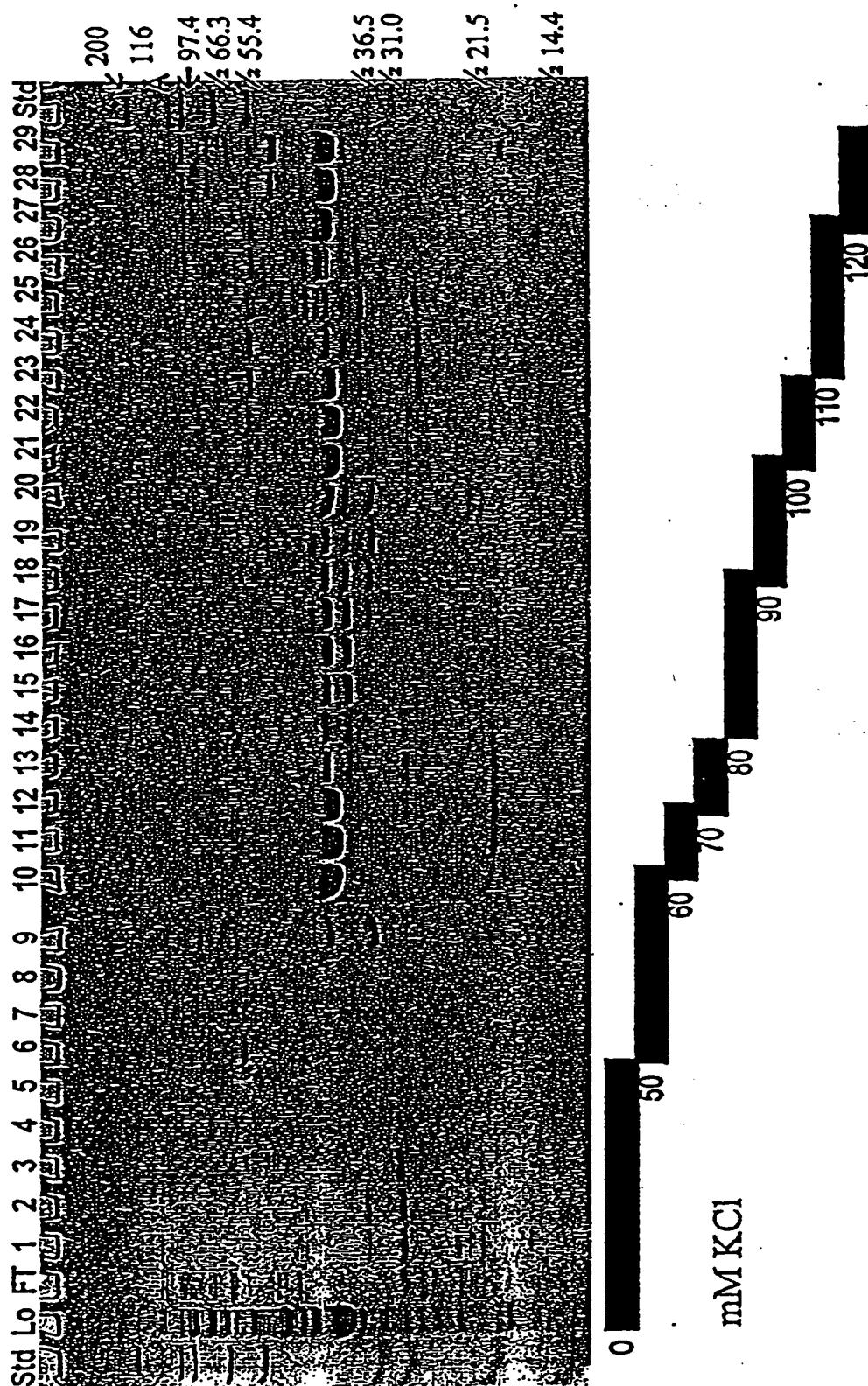


Fig. 19

20/49

DEAE Sepharose Column Fractions:



21/49

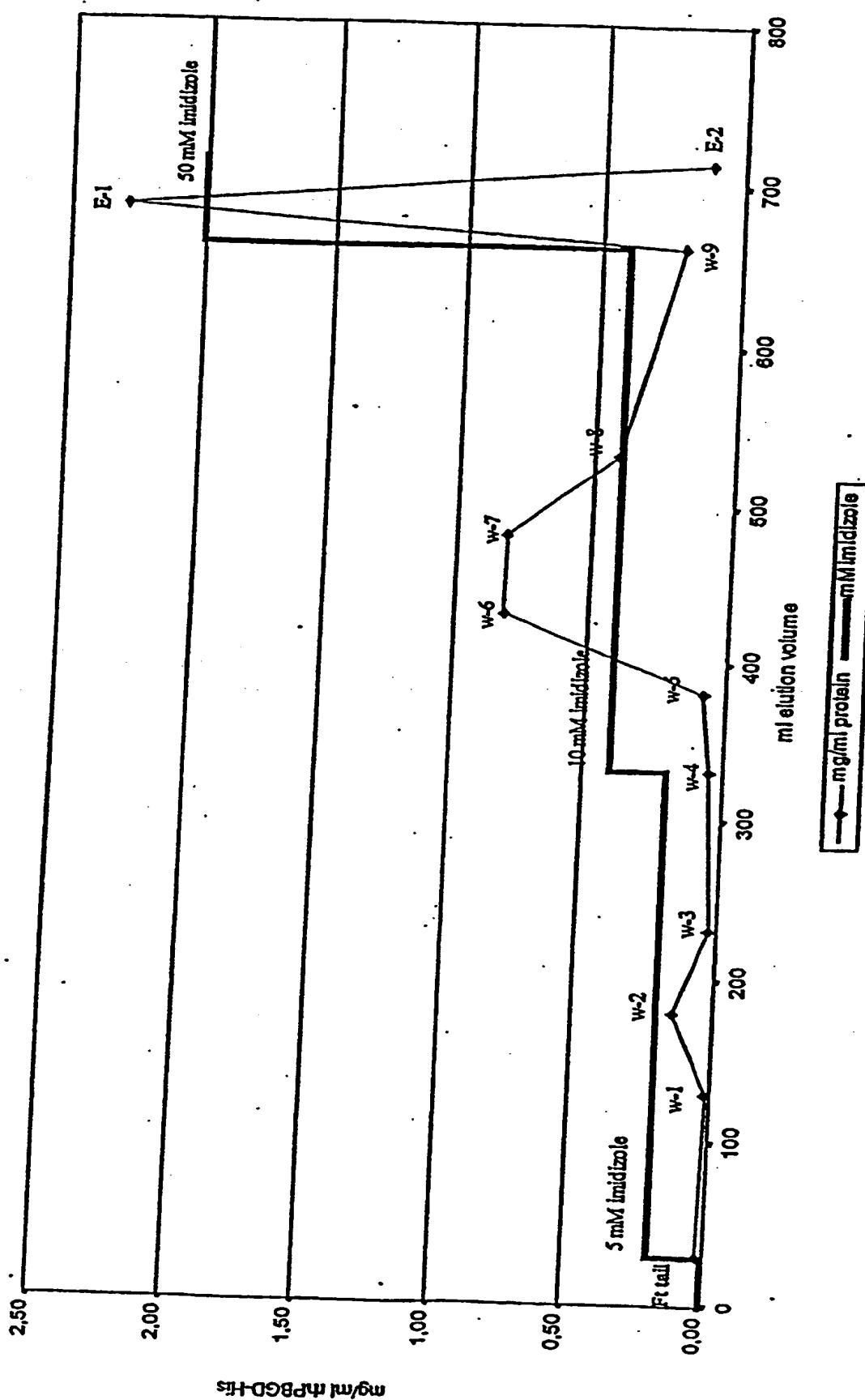


Fig. 21

22/49

1	Mark 12 molecular weight standards (Novex)
2	Cobalt load #1 (DEAE fractions 9 > 12)
3	Flowthrough from cobalt load #1
4	50 mM imidazole eluate from cobalt run #1
5	Concatamericized nickel purified rhPBGD-His
6	Flowthrough from cobalt load #2
7	10 mM imidazole eluate from cobalt run #2
8	50 mM imidazole eluate from cobalt run #2

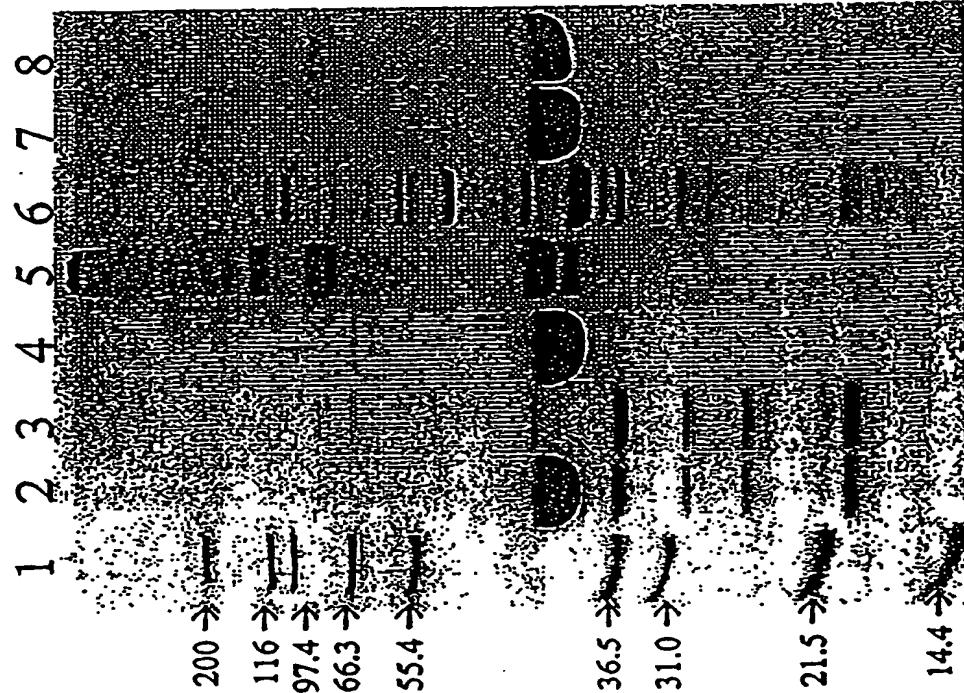


Fig. 22

23/49

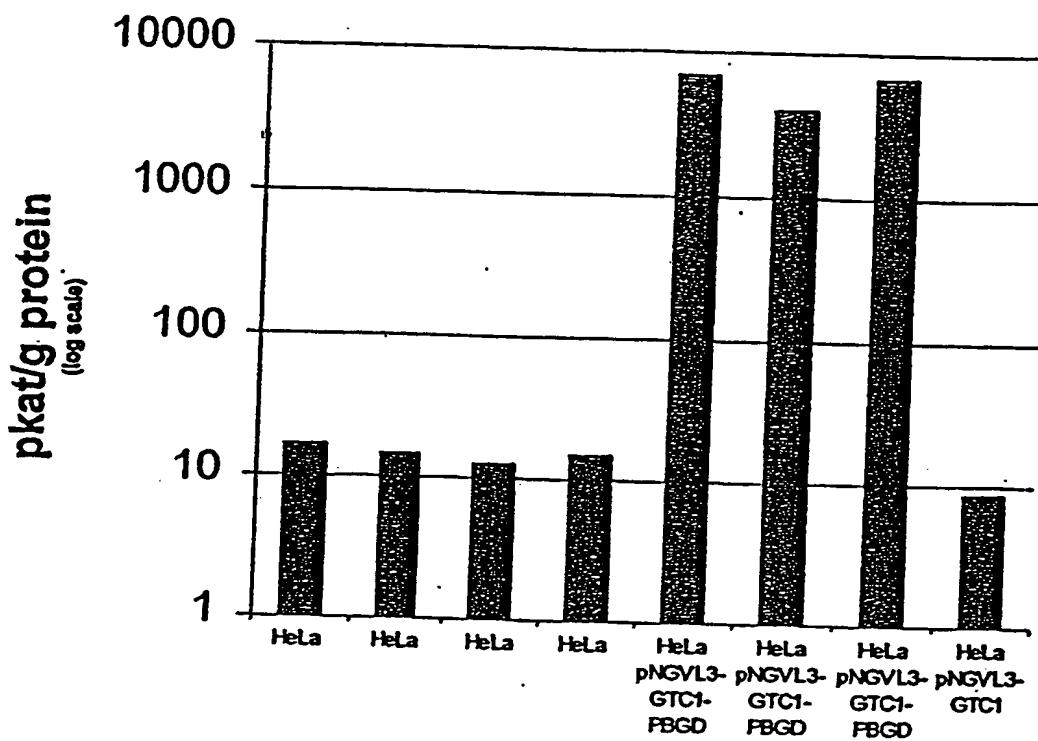


Fig 23: PBGD activity related to protein concentration in HeLa cells.

Fig. 23

24/49

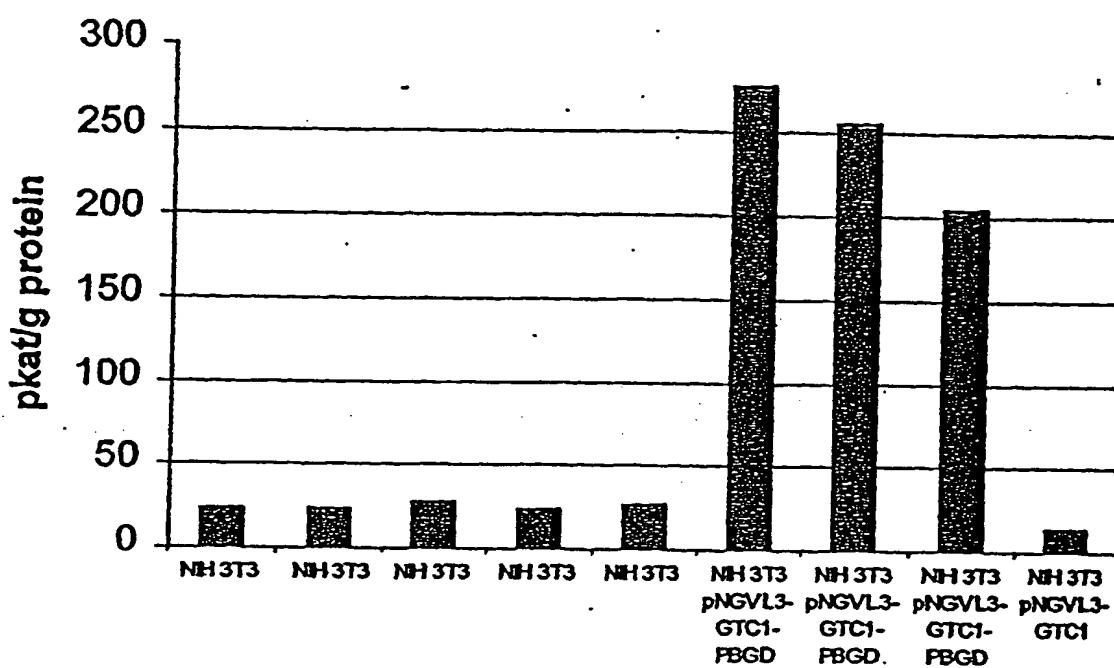


Figure 24: PBGD activity related to protein concentration in NIH 3T3 cells.

Fig. 24

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25/49

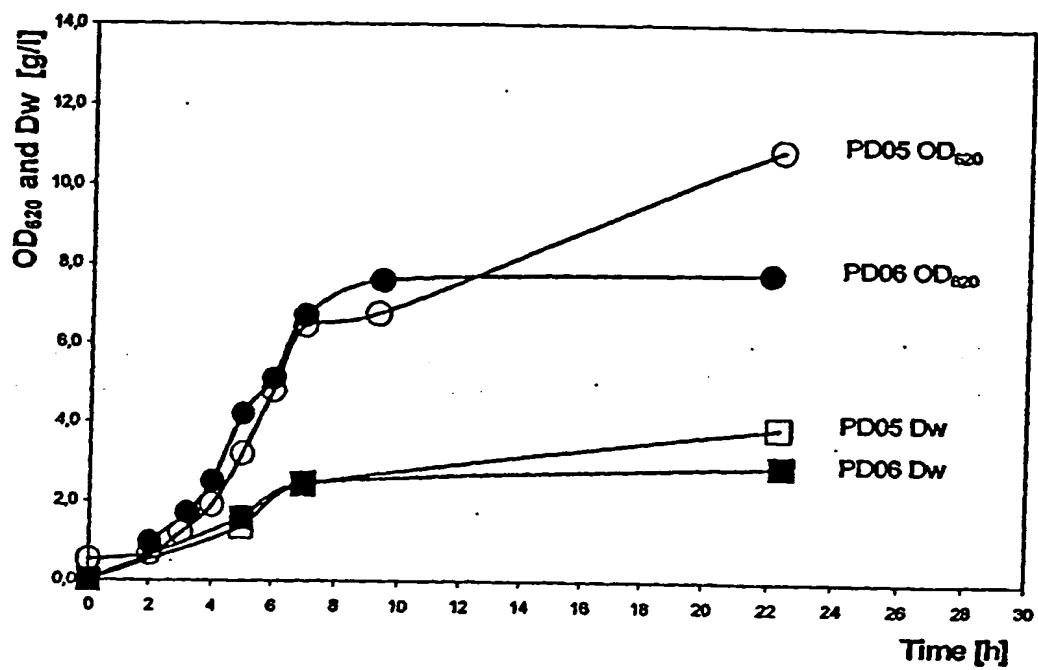


Fig 25. Comparison of fermentations PD05 and PD06 with strain PBGD-2.

Fig. 25

26/49

Fig 26. Comparison of fermentations PD09, PD11 and PD12

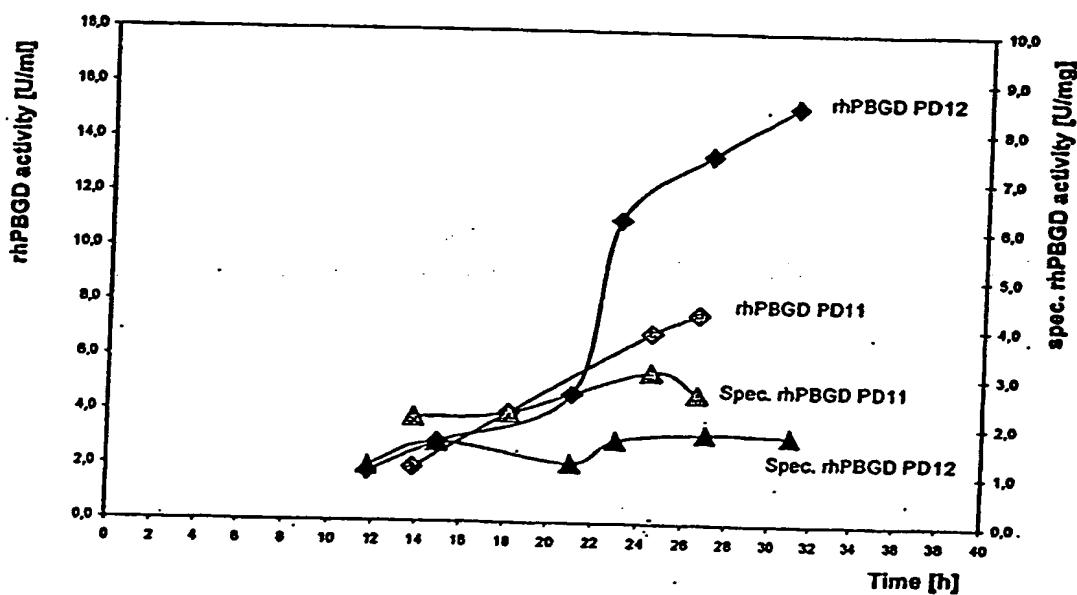


Fig. 26

27/49

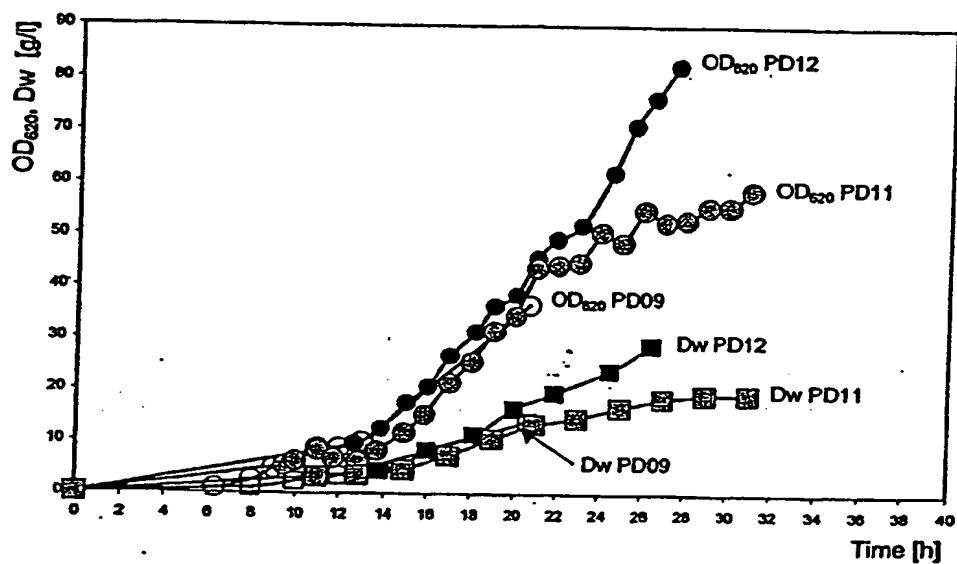


Fig 27: Comparison of fermentations PD09, PD11 and PD12 with strain PBGD-1.

Fig. 27

28/49

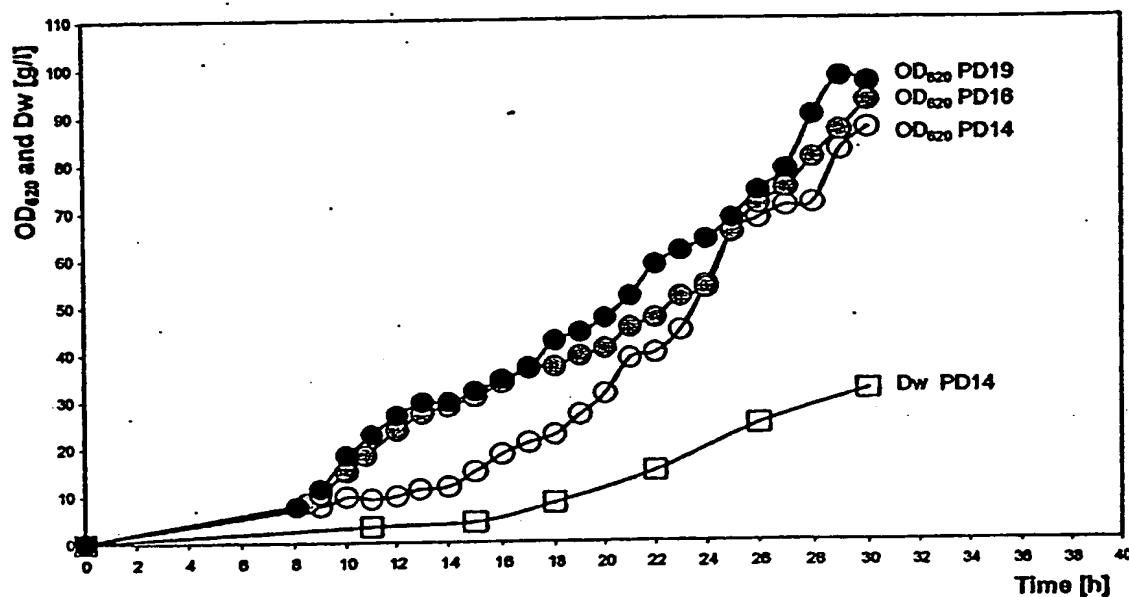


Fig 28.Comparison of fermentations PD14, PD16 and PD19 with strain PBGD-2.

Fig. 28

29/49

Fig 29.Comparison of fermentations PD14, PD16 and PD19 with strain PBGD-2.

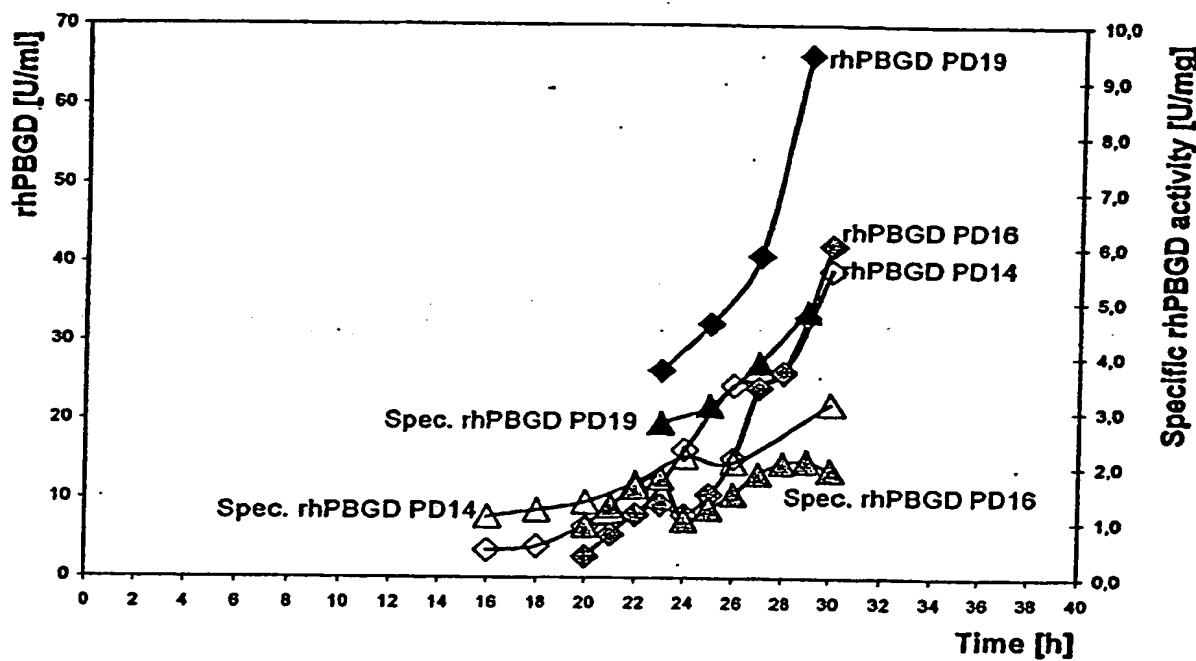


Fig. 29

30/49

Fig 30. Comparison of fermentations PD19, PD21 and PD22 with strain PBGD-2.

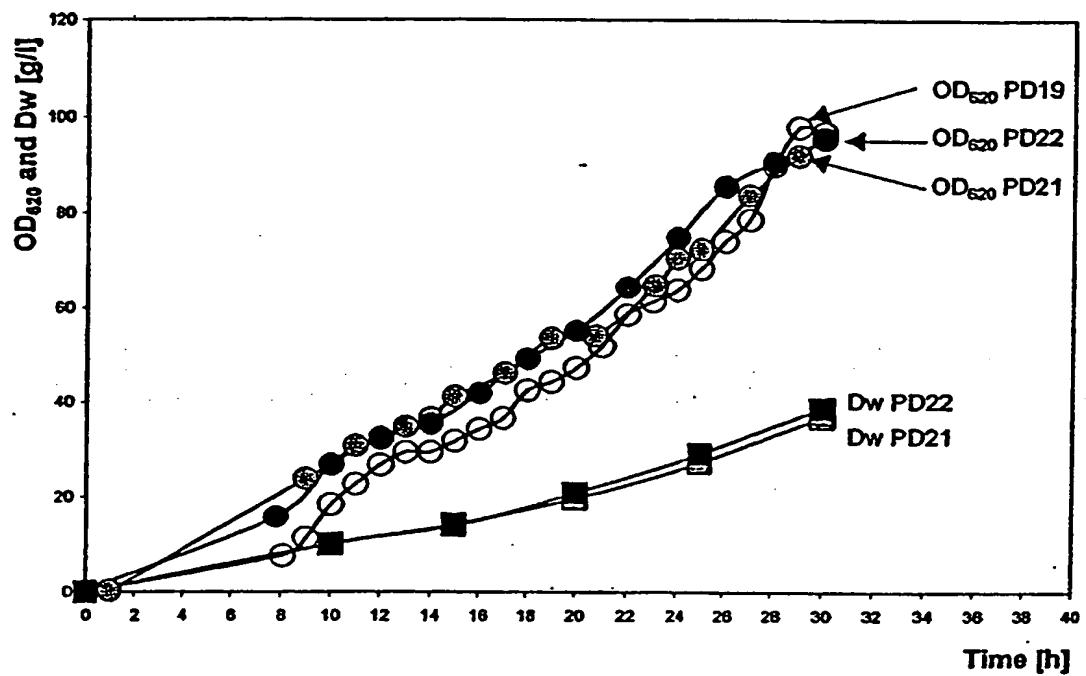


Fig. 30

31/49

Fig 31. Comparison of fermentations PD19, PD21 and PD22 with strain PBGD-2.

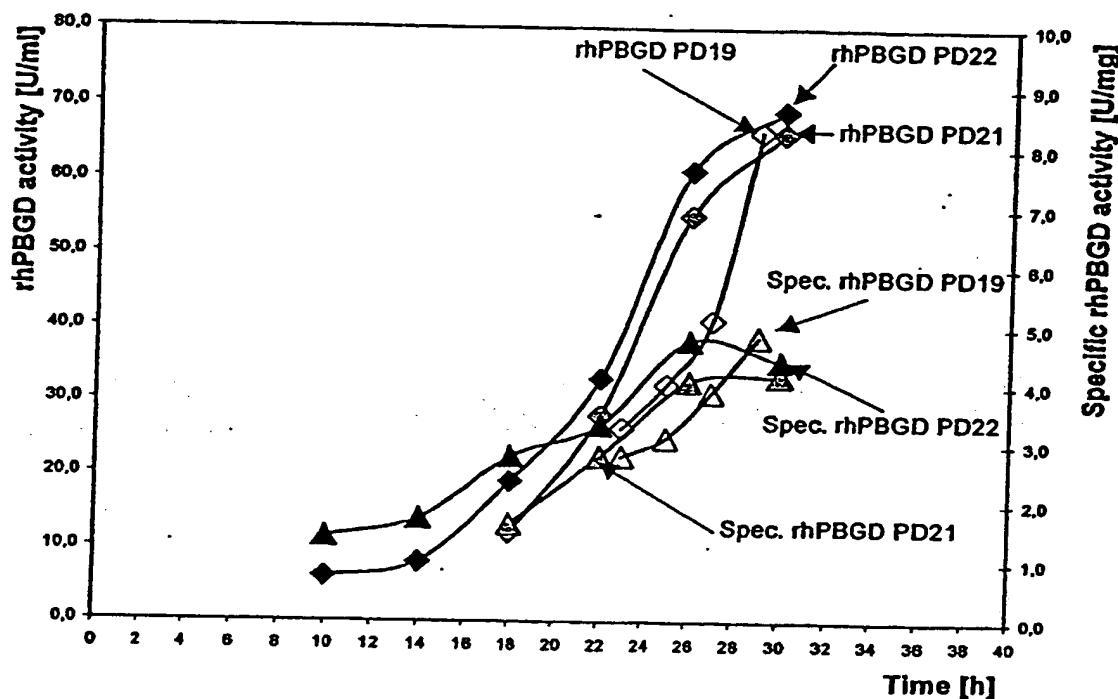


Fig. 31

32/49

Fig 32. Comparison of fermentations PD19, PD1501 and PD1502.

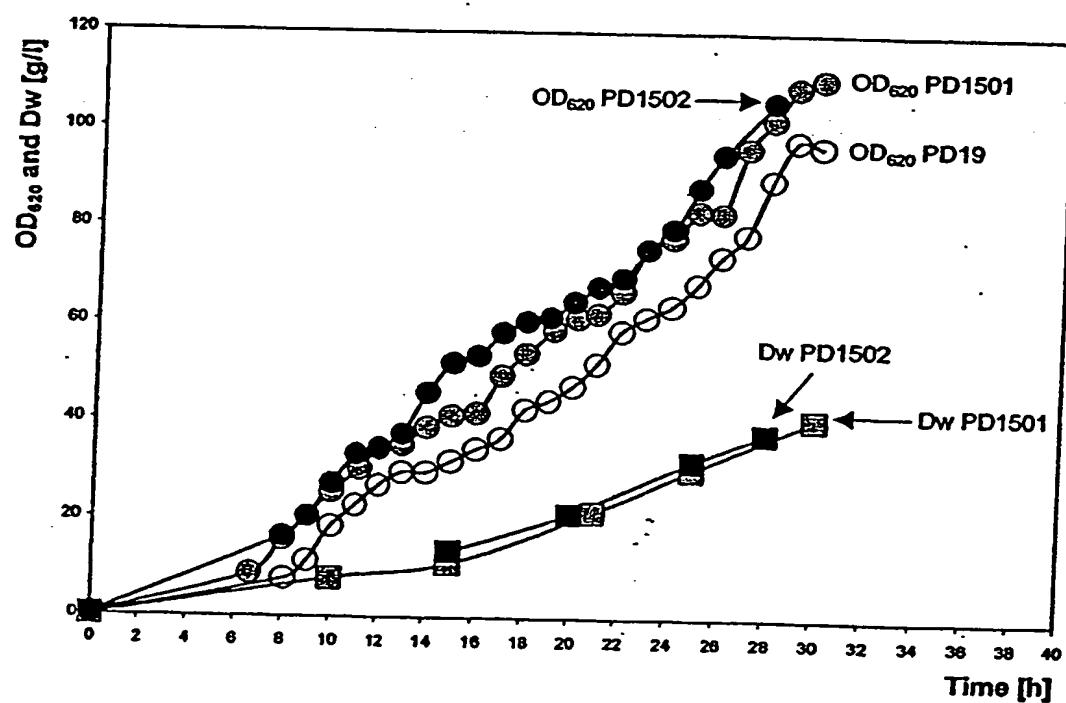


Fig. 32

33/49

Fig 33. Comparison of fermentations PD19, PD1501 and PD1502 with strain PBGD-2.

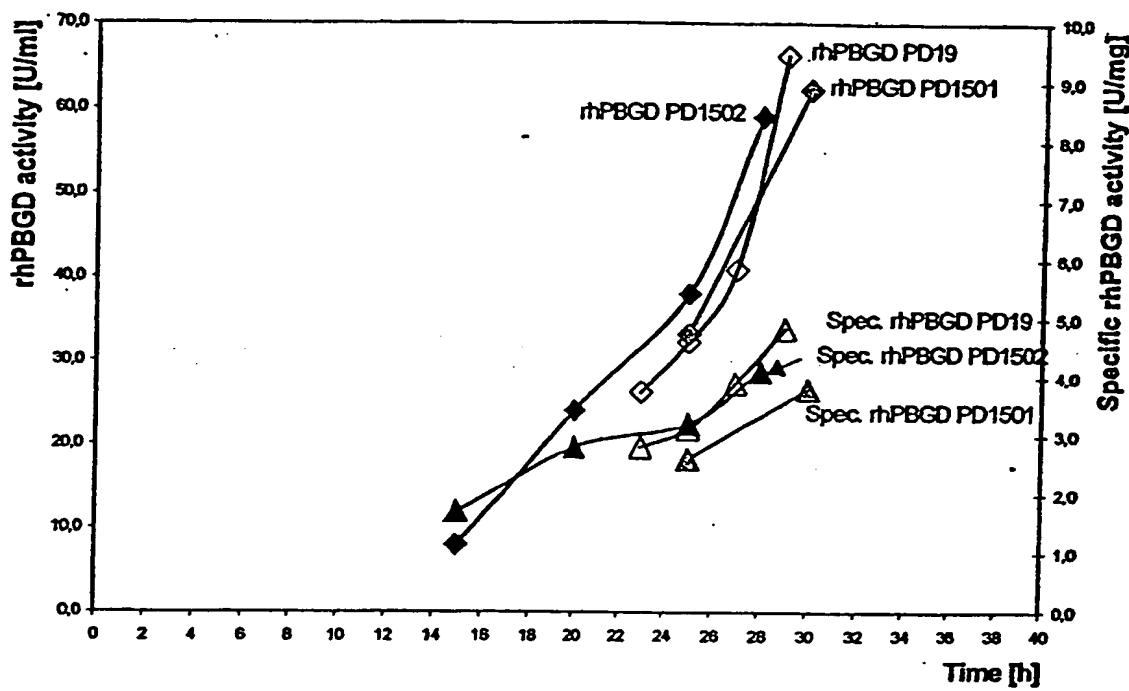
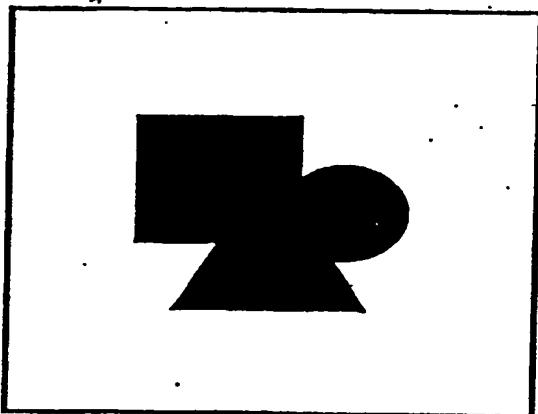


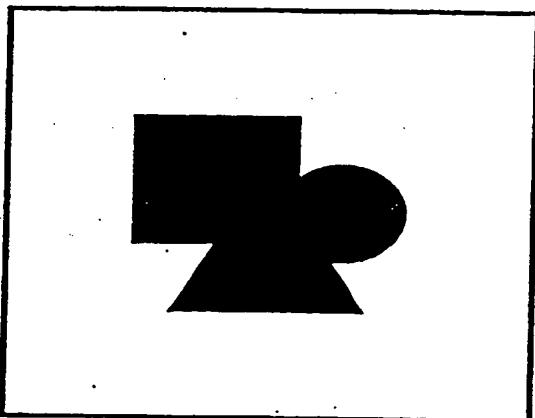
Fig. 33

34/49

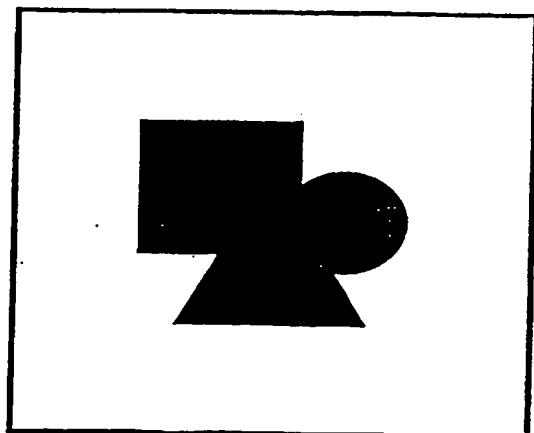
Summary of Fermentation and down stream process visualized by SDS-Page.
Comparison between different samples.



Well	Samples from PD22
1	Mark 12 MW st
2	Extract
3	Homogenate
4	Broth 30 h
5	Broth 26 h
6	Broth 22 h
7	Broth 18 h
8	Broth 14 h
9	Broth 10 h
10	rhPBGD-His



	Samples from PD1501
1	Mark 12 MW st
2	Extract
3	Extract
4	Extract
5	Broth 30 h
6	Broth 30 h
7	Broth 25 h
8	Broth 20 h
9	Broth 15 h
10	rhPBGD-His

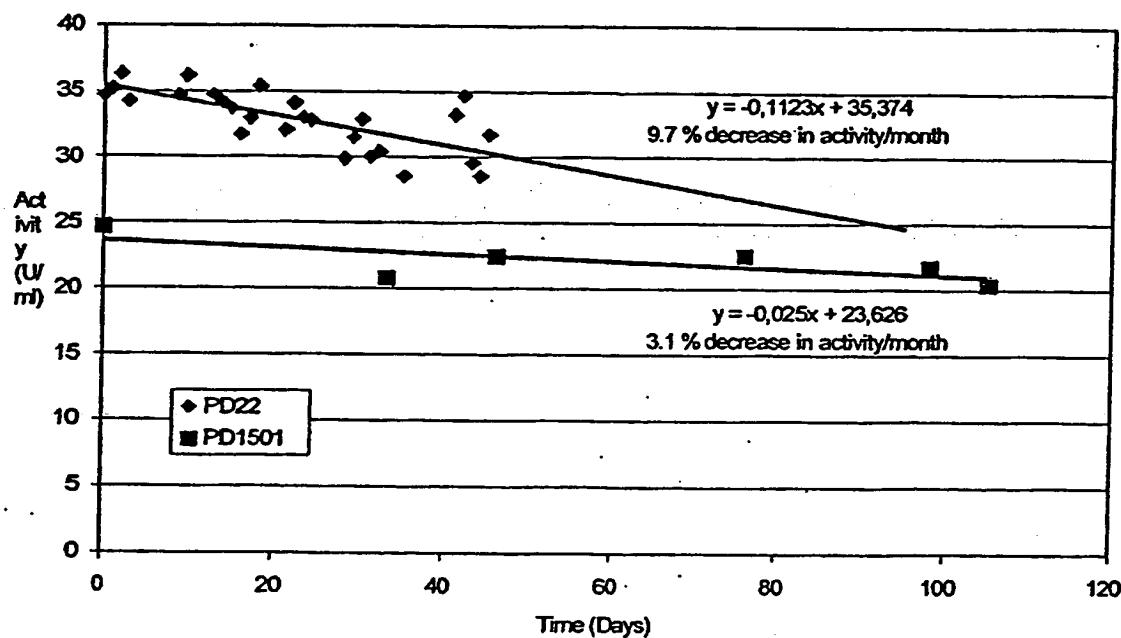


	Samples from PD1502
1	Mark 12 MW st
2	Broth 15 h
3	Broth 20
4	Broth 25 h
5	Broth 28 h
6	Homogenate
7	Extract
8	Extract
9	Extract
10	rhPBGD-His

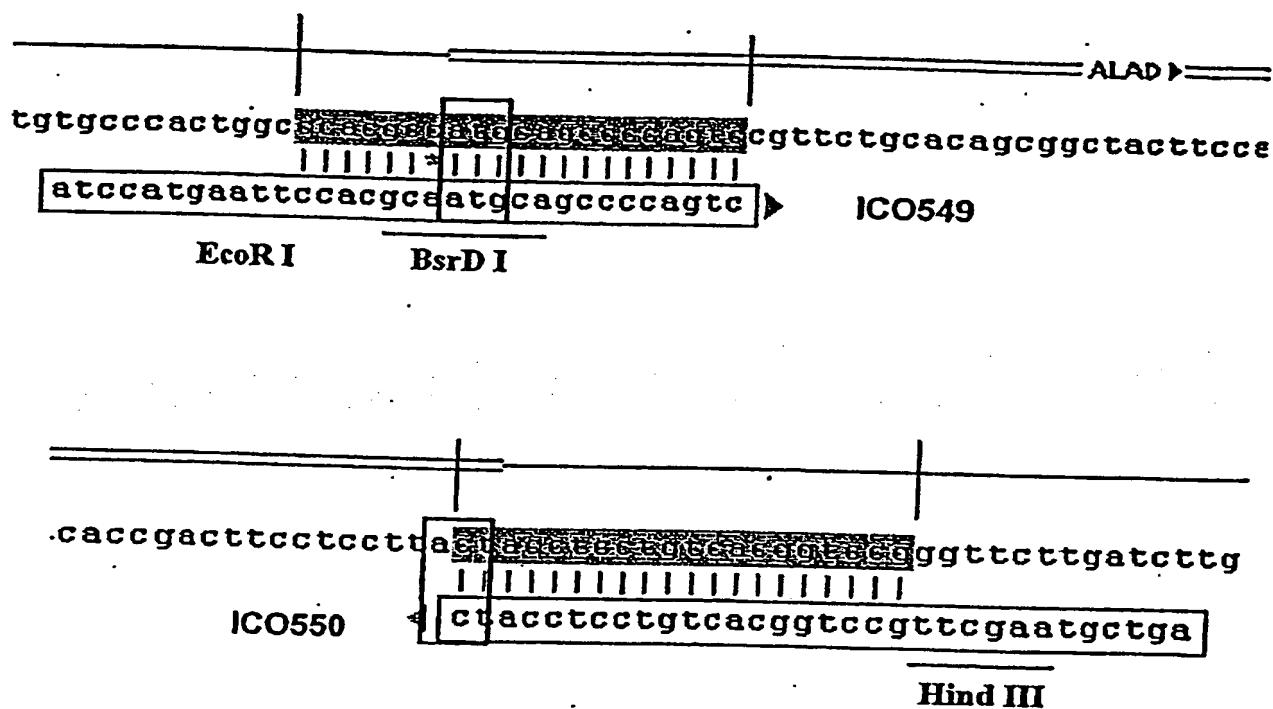
Fig. 34

35/49

Fig 35. Stability studies: Single use aliquots of extract were routinely taken out of the freezer (-20°C) and the rhPBGD-activity was measured and plotted over time.

**Fig. 35**

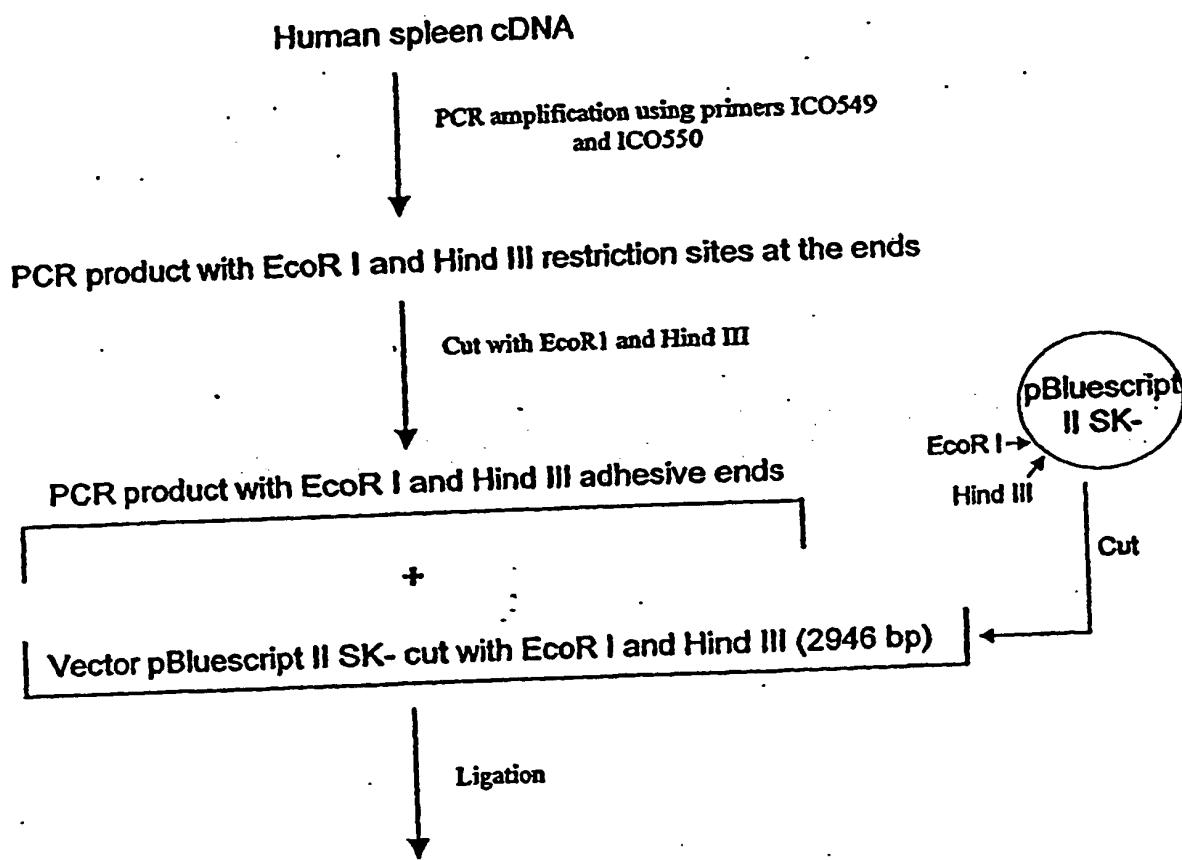
36/49

Description of the oligos used for PCR amplification

The two oligos ICO549 and ICO550 are shown below the ALAD sequence. The highlighted regions in the ALAD sequence represent the regions of homology with the oligos. The asterisk denotes the C to A change introduced to generate a *BsrD* I site for ease of manipulation of the 5' end of the cloned molecule. The initiation and termination triplets in the ALAD sequence are boxed. Both the oligos have 5' sequences extending beyond the regions of homology to incorporate restriction sites for cloning of the amplified DNA, *viz.* *EcoR* I for ICO549 and *Hind* III for ICO550.

Fig. 36

37/49

Strategy for PCR cloning of ALAD**Fig. 37**

38/49

ART 34 AMDT

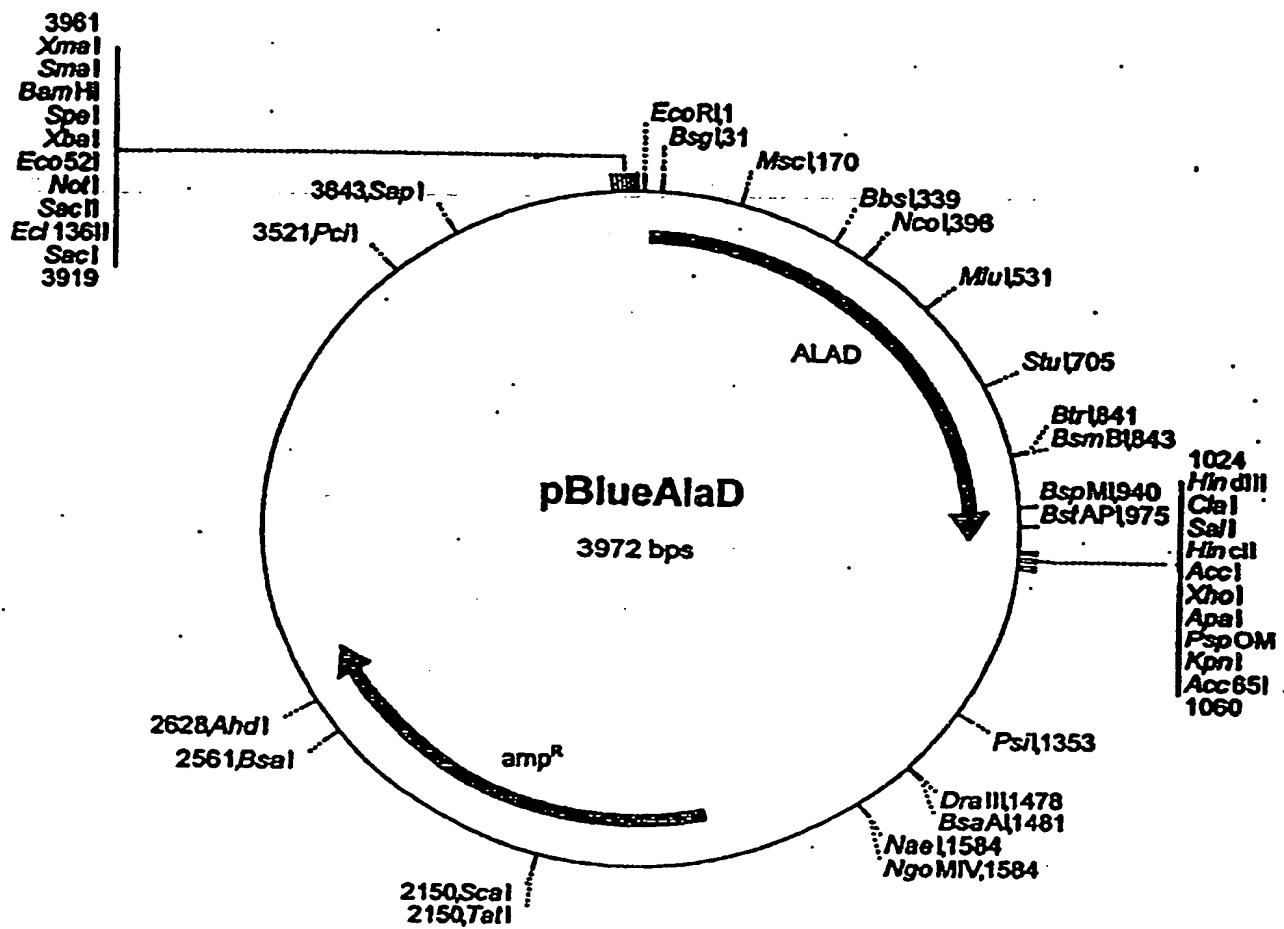


Fig. 38 37 C

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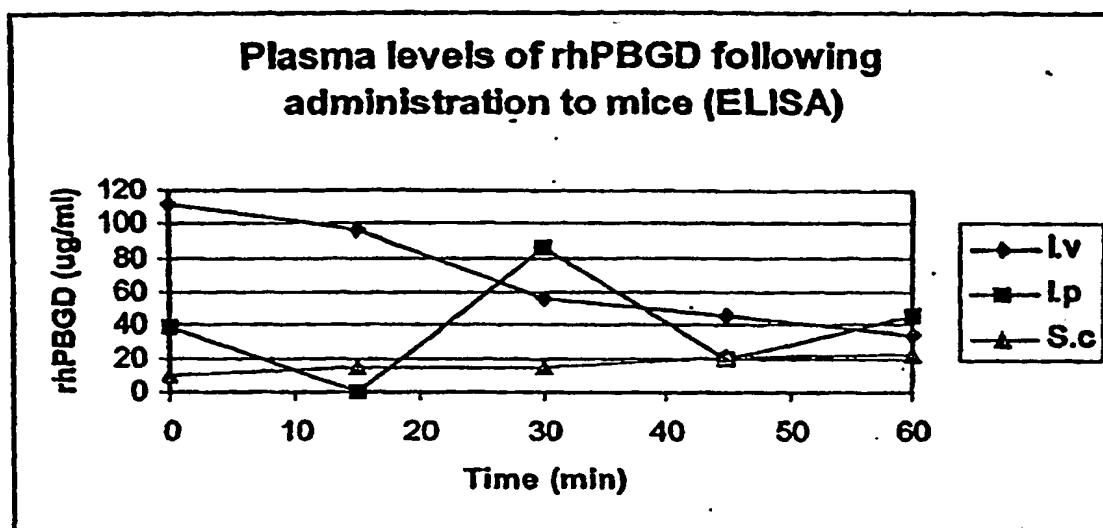
39/49**ART 34 AMDT**

Figure 38: Plasma levels of rhPBGD following administration to mice.

Fig. 39**SUBSTITUTE SHEET (RULE 26)**

WO 01/07065

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40/49

ART 34 AMDT

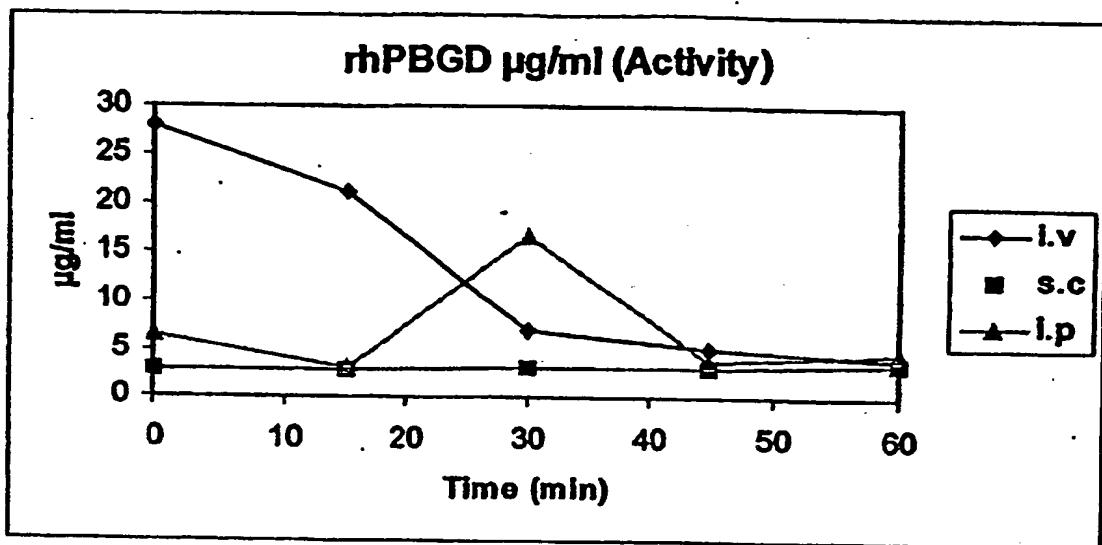


Figure 39: PBGD enzymatic activity in plasma following rhPBGD administration to mice

~~Fig. 40~~

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41/49

ART 34 AMDT

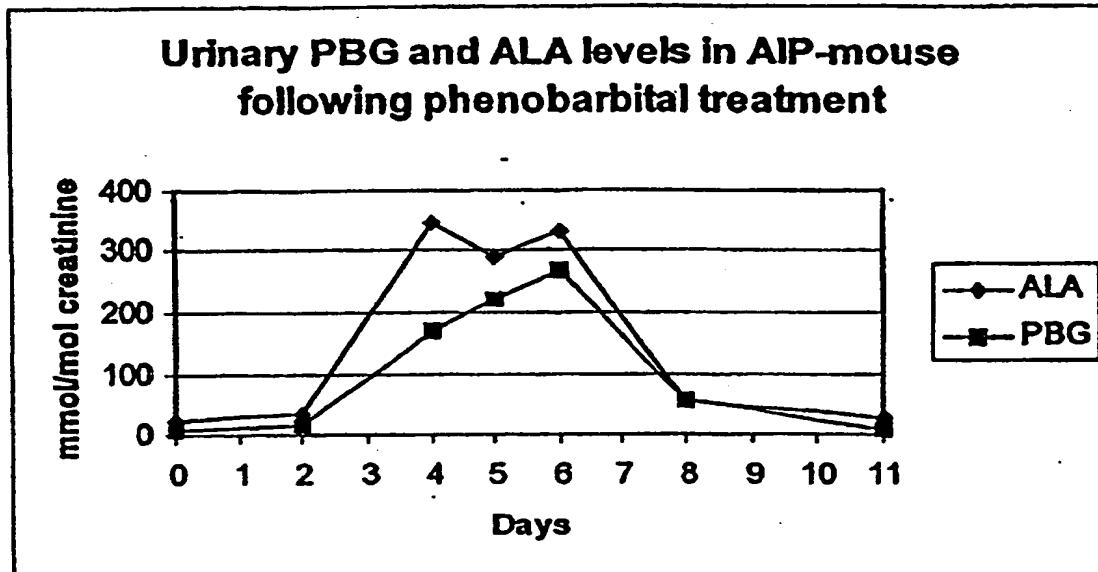


Figure 40: Urinary content of PBG and ALA in AIP-mouse treated with phenobarbital.

Fig. 41

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42/49

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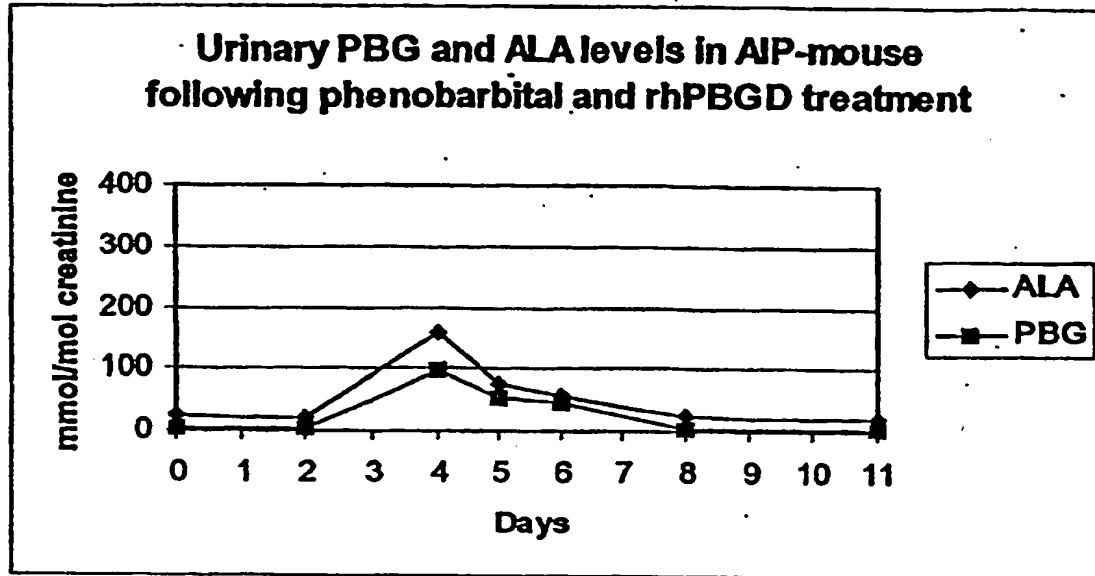


Figure 41: Urinary content of PBG and ALA in AIP-mouse treated with phenobarbital and rhPBGD. Mice were treated with an increasing dose of phenobarbital for 4 days (day 0-4,

Fig. 42

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43/49

ART 34 AMDT

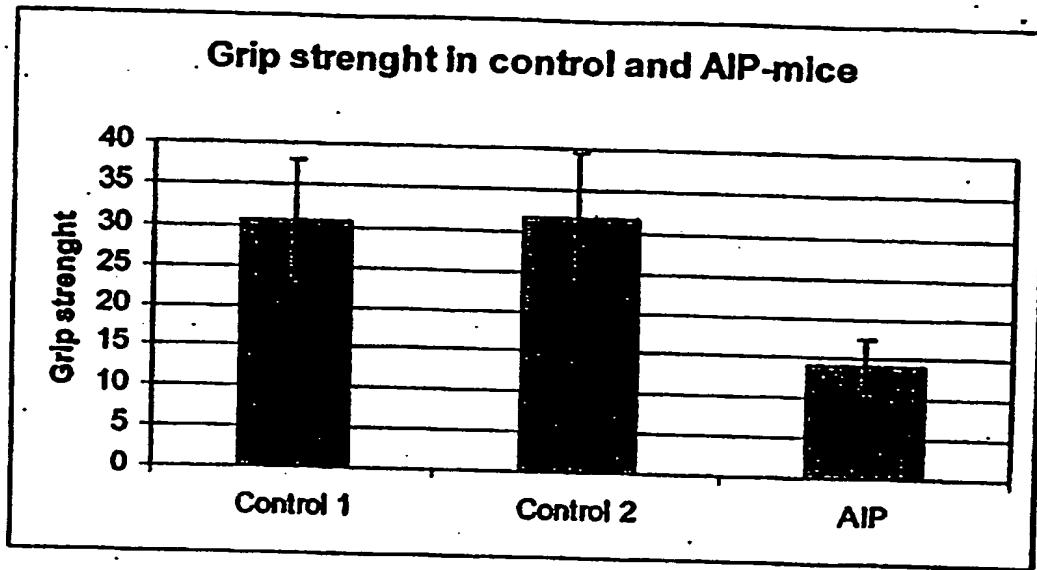


Figure 42: Grip strength analysis in control and AIP-mice. Grip strength were determined using a grip strength meter (Ugo Basile) in heterozygous control animals (control 1, n=5), in wild type controls (control 2, n=5) and in AIP-transgenic mice (AIP, n=5).

Fig. 43

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44/49

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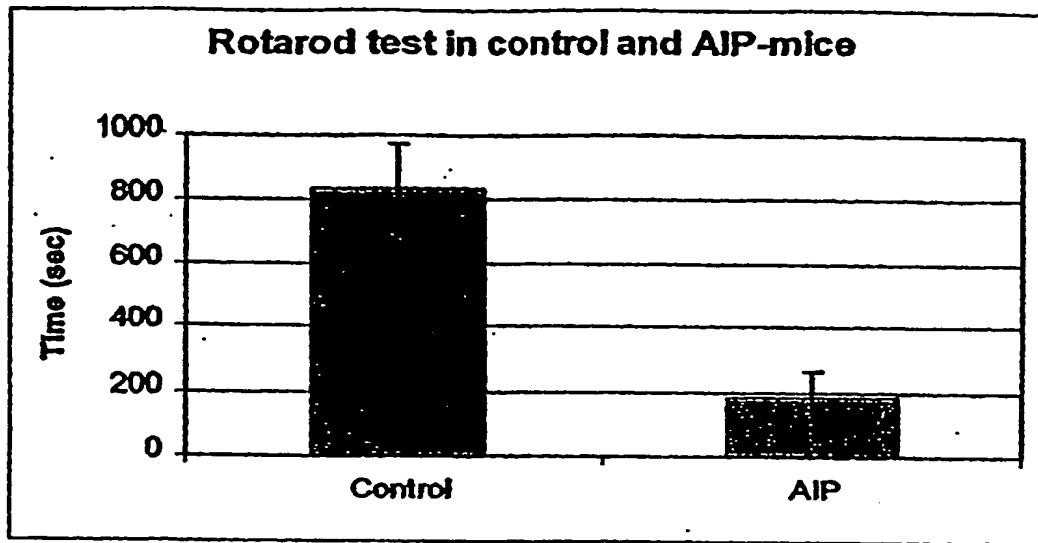


Figure 4 Rotarod analysis in control and AIP-mice. The rotarod analysis were determined using a rotarod treadmill (Ugo Basile) in wild type controls (control, n=5) and in AIP-transgenic mice (AIP, n=7).

Fig. 44/43

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45/49

ART 34 AMDT

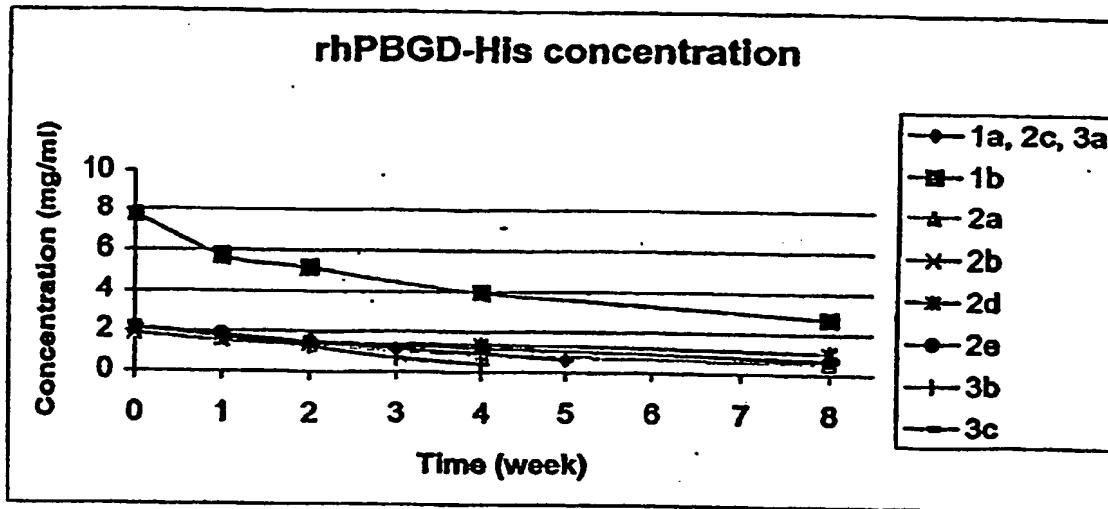


Figure 44. Enzyme concentration over 8 weeks at 40°C measured by HPLC. A decrease from 2 mg/ml to 0,5 mg/ml and 8 mg/ml to 2,5 was detected.

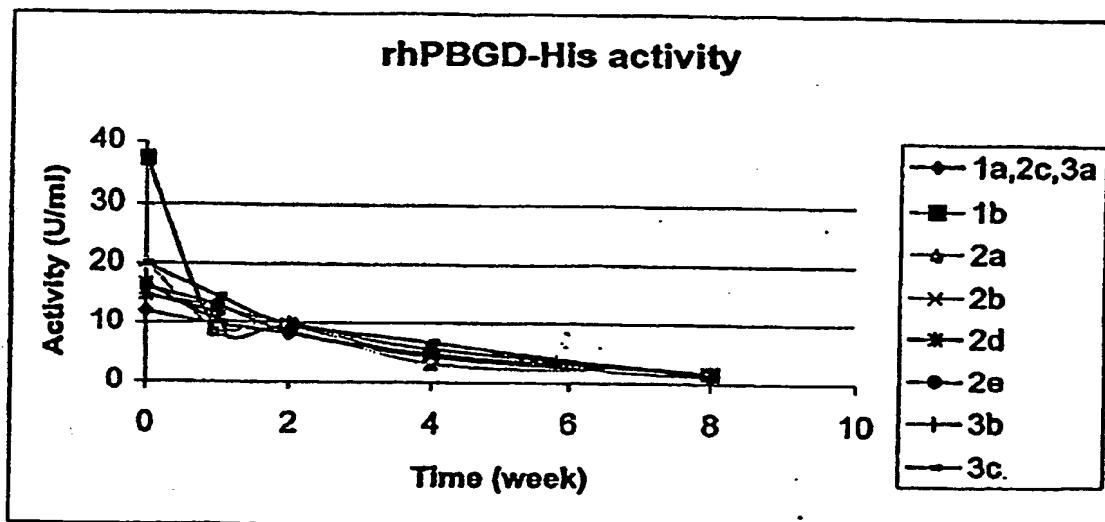


Figure 45. The enzyme activity measured over 8 weeks at 40°C. A significant decrease over the first week was seen for the high concentration sample, 1b. After two weeks the decrease rate was the same for all samples.

~~Fig. 45~~

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WO 01/07065

PCT/DK00/00425

46/49

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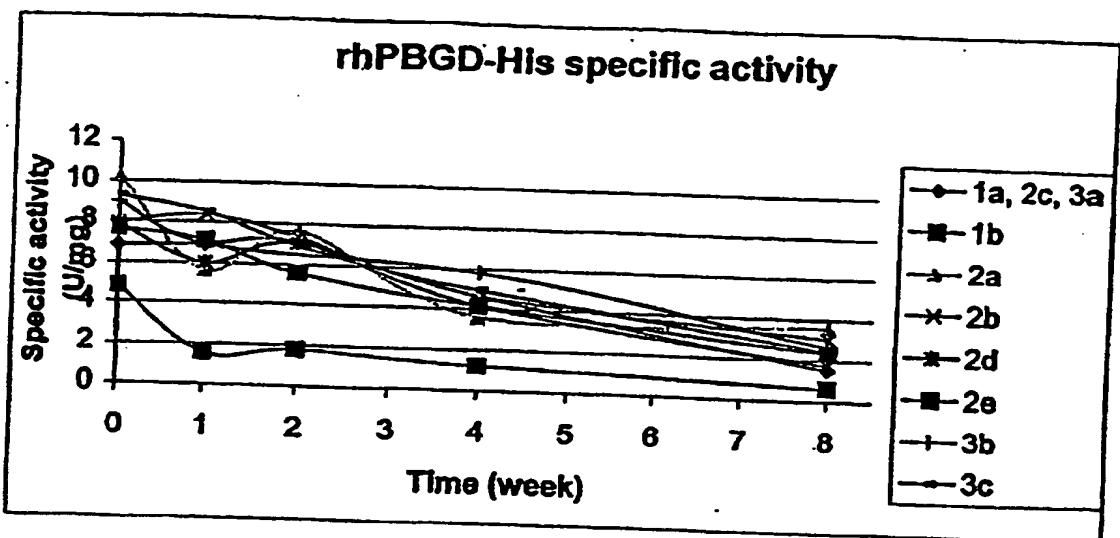


Figure 46. Enzyme specific activity measured during 8 weeks at 40°C. The activity was measured using the enzyme activity assay and the protein concentration was measured using HPLC.

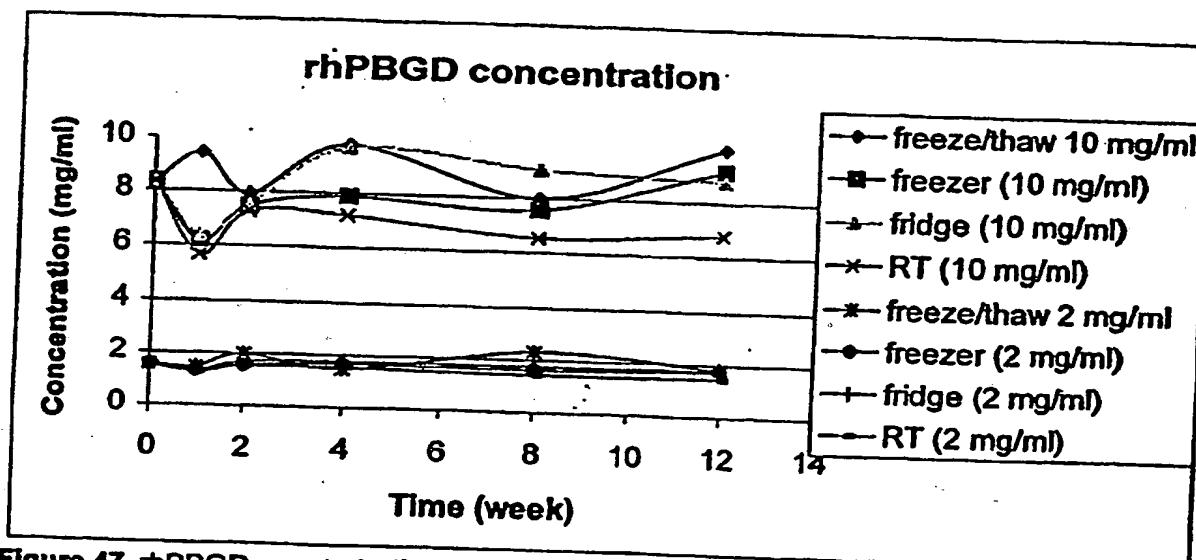


Figure 47. rhPBGD concentration over 12 weeks at -20°C (freezer), 5°C (fridge), 25°C (RT) and freeze/thawed at each sampling. The measurement was performed using HPLC.

~~Fig. 46~~

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WO 01/07065

PCT/DK00/00425

47/49

ART 34 AMDT

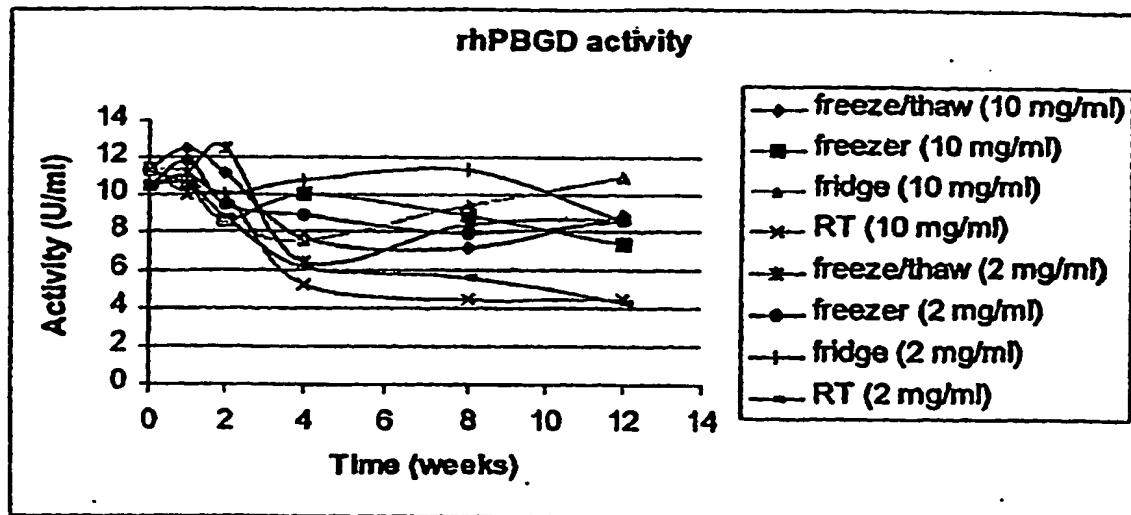


Figure 48. rhPBGD activity over 12 weeks at -20°C (freezer), 5°C (fridge), 25°C (RT) and freeze/thawed at each sampling.

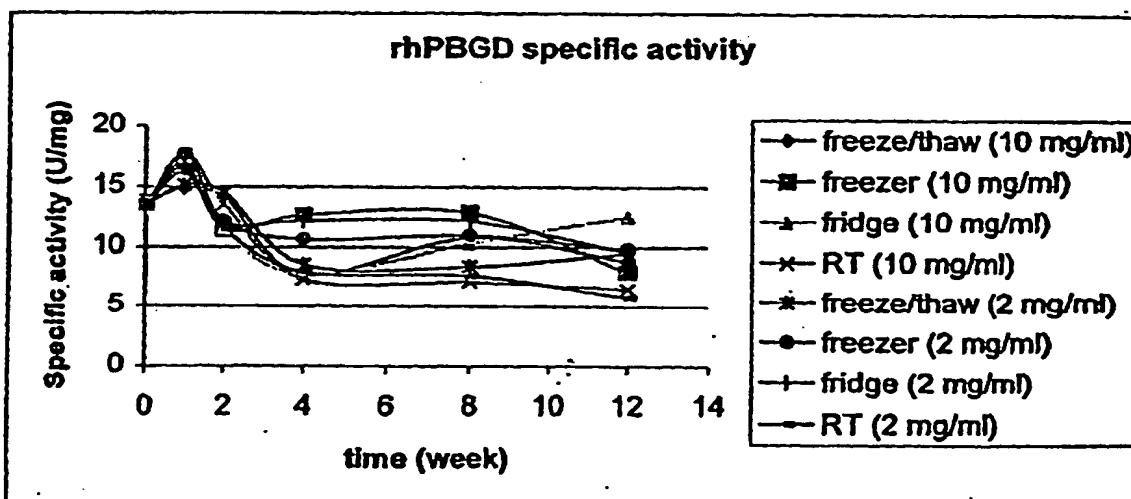


Figure 49. rhPBGD specific activity over 12 weeks at -20°C (freezer), 5°C (fridge), 25°C (RT) and freeze/thawed at each sampling. Measurements were performed using enzyme activity assay and HPLC.

~~Fig. 47~~

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WO 01/07065

PCT/DK00/00425

48/49

ART 34 AMDT

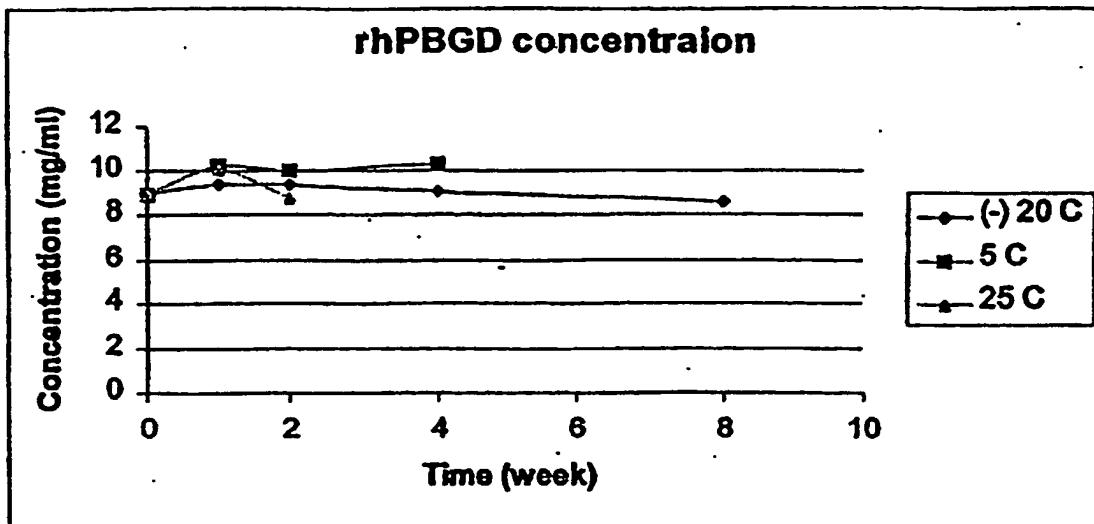


Figure 50. rhPBGD concentration measured over 8 weeks using BCA.

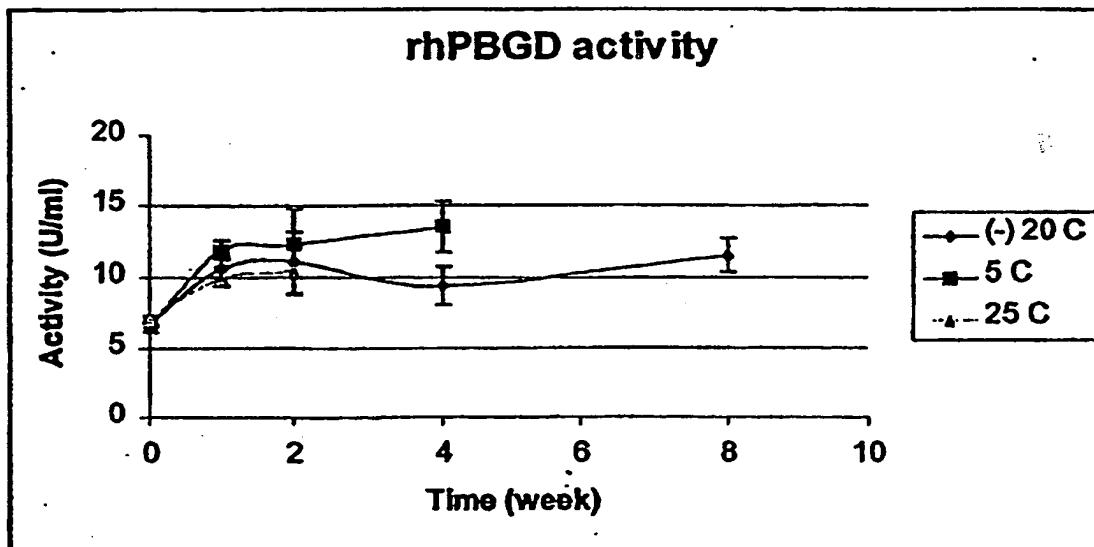


Figure 51. The rhPBGD activity measured over 8 weeks. The stability study has been performed under nitrogen at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Fig. 48

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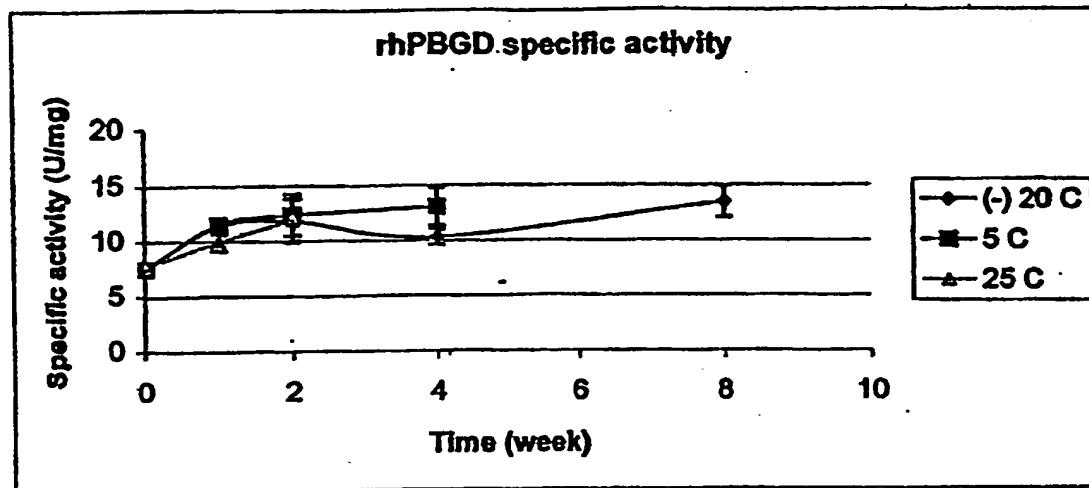
49/49**ART 34 AMDT**

Figure 52. The specific rhPBGD activity measured using the enzyme activity assay and BCA protein concentration assay. The stability study has been performed under nitrogen at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Fig. 49**SUBSTITUTE SHEET (RULE 26)**